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Note

Separation and determination of the hydrolysis products of chloramphenicol in pharmaceutical preparations by high-performance liquid chromatography

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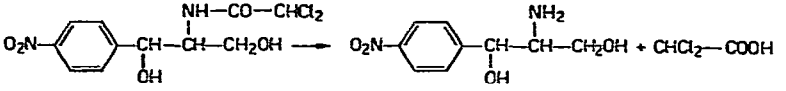
Chloramphenicol is a widely used antibiotic, and 2-amino-1-(*p*-nitrophenyl)-1,3-propanediol (I) commonly occurs as a hydrolysis product in pharmaceutical preparations. Other hydrolysis products which may develop are nitrobenzyl alcohol (II) and nitrobenzaldehyde (III). The specific determination of chloramphenicol by spectrophotometric or polarographic methods is difficult owing to the structural similarities of other contaminants. It is desirable to have an assay method for chloramphenicol alone, as it possesses the major microbiological activity.



Chloramphenicol

2-Amino-(p-nitrophenyl)-1,3-propanediol

(I)

A polarographic determination after thin-layer chromatographic (TLC) separation has been described recently[1]. There have been reports on the separation of chloramphenicol intermediates by high-performance liquid chromatography (HPLC). Several contaminants formed during synthesis have been separated and analysed by HPLC[2–4].

In this work, the hydrolysis products I, III and nitrobenzene (IV) were separated from chloramphenicol on a C_{18} reversed-phase column with a methanol–water–acetic acid solvent system. This system allows an adequate resolution and trace-level determination of impurities in 10–15 min. The major hydrolysis products I was determined in a number of dosage forms such as dragées, capsules, suppositories and ear- and eye-drops.

EXPERIMENTAL

All chemicals and solvents used were of reagent grade. The determination was carried on a Perkin-Elmer 1220 liquid chromatograph with a fixed-wavelength UV detector (254 nm) and a 1-mV revorder. A C_{18} reversed-phase column (0.25 m × 2.6 mm I.D.) packed with silica gel SI 100 (10 μm), coated with chemically bonded C_{18} organic phase, was used for separation. The mobile phase was water–methanol–

acetic acid (55:45:1). The contents of chloramphenicol and hydrolysis product were calculated by comparison with external standards in the same range of concentrations. The hydrolysis product 2-amino-1-(*p*-nitrophenyl)-1,3-propanediol was prepared by treating chloramphenicol with 0.1 *N* sodium hydroxide solution according to the method of Rebstock *et al.*[5].

All solid dosage forms, capsules, dragées and suppositories were extracted with methanol, filtered and made up to a suitable volume. Liquid dosage forms such as ear- and eye-drops were weighed accurately and diluted with methanol to the desired volume.

RESULTS AND DISCUSSION

A good separation of chloramphenicol from its major hydrolysis product (I) was achieved. Nitrobenzaldehyde (III) and nitrobenzene (IV) were also well separated (Fig. 1).

Nitrobenzyl alcohol overlapped with the chloramphenicol peak, but was not present in the pharmaceutical preparations. The products III and IV were also not

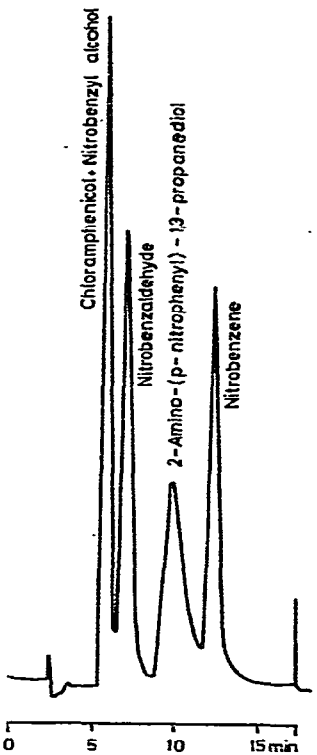


Fig. 1. HPLC separation of chloramphenicol and other hydrolysis products. Column, C_{18} reversed-phase column (0.25 m × 2.6 mm I.D.) packed with silica gel SI 100 (10 μm); flow-rate, 1 ml/min; wavelength, 254 nm; mobile phase, water–methanol–acetic acid (55:45:1); temperature, ambient; amount injected, 5 μl; pressure, 1200 p.s.i.

detected. This was checked by TLC using a silica gel GF 254 plate with acetone–benzene–light petroleum (b.p. 40–60°) (2:2:1) as mobile phase. Compound I was found in different pharmaceutical preparations at levels in the range 0.05–1.5%. Two formulations contained I at concentrations as high as 11.5 and 29.2%.

An optimal separation was obtained under isocratic conditions by adjustment of the methanol content of the aqueous eluent. The resolution and retention are governed by the concentration of methanol in the mobile phase. The presence of about 1% of acetic acid improved the elution. The k' values obtained for chloramphenicol and the products I, III and IV were 1.35, 2.95, 1.85 and 3.95, respectively. The selectivity coefficient, α, for the major hydrolysis product I with respect to chloramphenicol was 2.18. The separation deteriorated slightly if isopropanol was used in place of methanol. It has been reported[6] that up to $k' = 10$, solute interaction takes place with a monomolecular layer of solvent adsorbed on the surface of the bonded organic reversed-phase, and not with the hydrocarbon chain itself. The order of elution of chloramphenicol and the other four compounds, I, II, III and IV, is governed by their structural characteristics and seems to be in agreement with this hypothesis. Highly polar nitrobenzene, with a dipole moment of 4.2 D, is strongly retained and eluted last. The selectivity is due only to solute–solvent interactions. If the retention mechanism was considered to be based on the interaction of the solute molecules with the hydrocarbon chain of the reversed phase, the highly polar nitrobenzene (4.2 D) and nitrobenzaldehyde (2.4 D) would have been eluted first.

There was a linear relationship between concentration and peak area in the range 10–100 μg/ml for chloramphenicol and the hydrolysis product I, and the detection limits were 5 and 10 μg/ml, respectively, for an injected volume of 5 μl. The coefficient of variation in this concentration range was $\pm 3.2\%$.

ACKNOWLEDGEMENT

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