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Note

Simplified thin-layer chromatographic method for the simultaneous determination of chloramphenicol and 1-(4'-nitrophenyl)-2-aminopropane-1,3-diol

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It is known[1,2] that chloramphenicol (I) loses its antibiotic activity by the hydrolysis of the amide bond to give 1-(4'-nitrophenyl)-2-aminopropane-1,3-diol (II). This latter compound commonly occurs in pharmaceutical preparations and, because of the structural similarity of I and II, the specific determination of I by spectrophotometry is unsuitable. It is desirable to have a rapid and accurate assay method for both I and II. Recently a polarographic determination, after thin-layer chromatography (TLC)[3] and separation by high-performance liquid chromatography (HPLC), has been described[4].

As an alternative, this note describes a simple and rapid method for the estimation of I and II. The principle of this method consists of spotting the solutions on a TLC plate together with standard solutions, and developing the plate with an ethyl acetate–formic acid–water (10:2:8, upper phase) solvent. The intensities of the various spots are then measured by a densitometer and peak heights of the standards are used to calculate the concentration of I and II in unknown samples.

EXPERIMENTAL

Reagents

All reagents and solvents were of analytical grade (Carlo Erba, Milan, Italy). Compound II was prepared by the acid hydrolysis of compound I[5].

The thin-layer developing solvent was an ethyl acetate–formic acid–water (10:2:8, upper phase) mixture.

Apparatus

A Vitatron densitometer (Model TLD 200) was used to measure the intensity of the spots.

Standard solutions

Compounds I and II were dissolved in methanol to yield solutions containing 1 mg of each compound per ml of methanol. By further appropriate dilutions, stock solutions containing 5–20 μg of I, and 0.5–2 μg of II, per 10 μl of methanol were prepared.

Sample solutions

Both solid and liquid dosage forms were weighed accurately and diluted with methanol to the desired volume ($C_I \approx 1$ mg/ml).

Spotting the plates

Standard solutions and sample solutions, alternating in duplicate, were applied to a silica gel GF_{254} (Merck, Darmstadt, G.F.R.) 20 × 20 cm plate.

Locating the spots

The solvent was allowed to migrate to a height of 10 cm above the point of application, whereupon the plate was dried in an oven at 110°. After cooling, the plate was scanned by UV light (250 nm); I and II could easily be discerned as dark spots. The lowest limit of detection that was possible, without losing accuracy, was 0.5 μg for both I and II.

RESULTS AND DISCUSSION

The mixture *n*-butanol–acetic acid–water (4:1:1) has been suggested for TLC separation[3] of I from its hydrolysis products. However, this chromatographic system was slow, whereas, in the developing solvent selected for this procedure, I and II move on silica gel plates rapidly and without trailing ($R_{F_I} = 0.9$; $R_{F_{II}} = 0.25$). When preservatives are present, such as the widely used *p*-hydroxybenzoate, they are easily separated from I and II by the same mixture to which diethyl ether is added in the ratio of 2:1.

The peak heights measured for the standards were used to plot a standard graph of peak height of I and II *versus* concentrations (Fig. 1). The concentration of samples of I and II could be obtained by interpolating this graph.

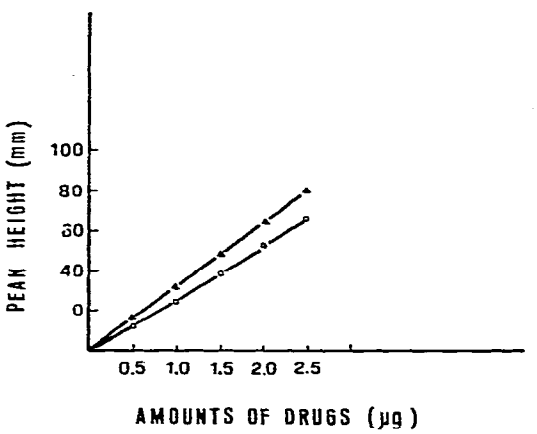

Fig. 1. Standard curves for I (▲) and II (○), expressed as peak height *versus* amount.

The accuracy and reproducibility of the method were determined by preparing samples containing known amounts of I and II and having these samples analyzed by a technician to whom the actual concentrations had not been revealed. The results were very satisfactory and are presented in Table I.

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE DETERMINATION OF I AND II

I (μg/ml)		*II (μg/ml)*	
Added	*Mean recovery ± S.D.**	*Added*	*Mean recovery ± S.D.**
5.5	5.33 ± 0.32	1.8	1.71 ± 0.25
12.5	12.42 ± 0.21	2.5	2.37 ± 0.31
31.4	30.7 ± 0.86	3.6	3.52 ± 0.36

* $n = 4$.

It can be concluded that TLC followed by densitometric measurement of the spots can be extremely useful for a rapid and quantitative recovery of both compounds I and II.

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