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Note

Determination of 4-chloroaniline and chlorhexidine digluconate by ion-pair reversed-phase high-performance liquid chromatography

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Chlorhexidine digluconate is widely used as an antibacterial agent in ophthalmic preparations and antiseptic contact lens solutions. Spectrophotometric methods have been utilized for the determination of chlorhexidine. Holbrook[1] developed such a procedure, but several components in the pharmaceutical preparations interfered in the determination. Chlorhexidine has previously been determined by a spectrophotometric method[2] involving ion pairing of the material with an acid dye and subsequent extraction into an organic phase. Other methods have included gas–liquid chromatography[3] and high-performance liquid chromatography (HPLC)[4].

The breakdown product, 4-chloroaniline has also been determined by HPLC[5]. The procedure presented here determines both chlorhexidine digluconate and 4-chloroaniline in the same one-step operation, which is suitable for stability studies of ophthalmic preparations and antiseptic contact lens solutions.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of two Waters Assoc. 6000A constant-flow pumps, a Waters Assoc. WISP 7103 automatic injector system with a 30-μl loop, a μBondapak C_{18} column (10 μm) (30 cm × 3.9 mm I.D.), a Schoeffel 750 detector set at 254 nm, a Waters Assoc. M730 data module and a Waters Assoc. M720 system controller.

The mobile phases were filtered through 0.22-μm filters and continuously degassed with helium.

Preparation of mobile phases

Sodium acetate–acetic acid buffer (pH 4.0). Weight exactly 2.72 g of sodium acetate trihydrate into a 1000-ml volumetric flask and add 600 ml of purified water (18 MΩ). Adjust the pH to 4.0 with 4.7 ml of acetic acid and adjust to volume with purified water (18 MΩ). Filter the solution immediately through a 0.22-μm filter.

Sodium heptanesulphonate solution. Weight out exactly 5 g of sodium heptanesulphonate salt into a 150-ml volumetric flask, add 20 ml of acetic acid and adjust to volume with purified water (18 MΩ).

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Mobile phase 1. This is a 92.1:0.2:4.8:2.9 mixture of buffer (pH 4.0), acetic acid, methanol and sodium heptanesulphonate solution.

Mobile phase 2. This is a 4.8:0.4:91.9:2.9 mixture of buffer (pH 4.0), acetic acid, methanol and sodium heptanesulphonate solution.

Preparation of standards

Place 308 mg of 4-chloroaniline in a 100-ml volumetric flask, and adjust to volume with methanol–purified water (18 MΩ) (50:50). Transfer 1 ml into a 100-ml volumetric flask and adjust to volume with purified water (18 MΩ). This stock solution has a concentration of 3.08 mg per 100 ml and 1/2, 1/4, 1/8, 1/16 and 1/20 dilutions are utilized for the calibration graph. Transfer 1 ml of a 20% chlorhexidine digluconate solution into a 100-ml volumetric flask and adjust to volume with purified water (18 MΩ). Dilute 5 ml of this solution to 100 ml with purified water (18 MΩ). This stock solution has a concentration of 20 mg per 100 ml and 1/2, 3/8, 1/4, 3/16, 1/8, 1/16, 1/32 and 1/64 dilutions are utilized for the calibration graph.

Chromatographic conditions

At the beginning of each day mobile phases continuously degassed with helium are pumped through the column for 1.5 h at 1.8 ml/min in order to establish stable baseline conditions. The UV detector is set at 254 nm.

Standards are chromatographed with the gradient shown in Table I. A gradient has to be used, because, the affinities of 4-chloroaniline and chlorhexidine digluconate for the column are very different, and because of the possible presence of interfering substances in pharmaceutical dosage forms (Fig. 1). The re-equilibration time is 3 min.

All the standards are injected five times successively.

TABLE I

MOBILE PHASE GRADIENTS USED

Time (min)	*Mobile phase 1 (%)*	*Mobile phase 2 (%)*	*Curve number of gradient*
0	55	45	–
6	20	80	6
10	20	80	6
12	55	45	6

RESULTS AND DISCUSSION

A typical routine chromatogram of 4-chloroaniline and chlorhexidine digluconate is shown in Fig. 2.

Assay of 4-chloroaniline

Assays were carried out with 4-chloroaniline solutions containing 1540, 770, 231, 154 and 77 μg per 100 ml. A 30-μl volume of each solution was injected five times successively.

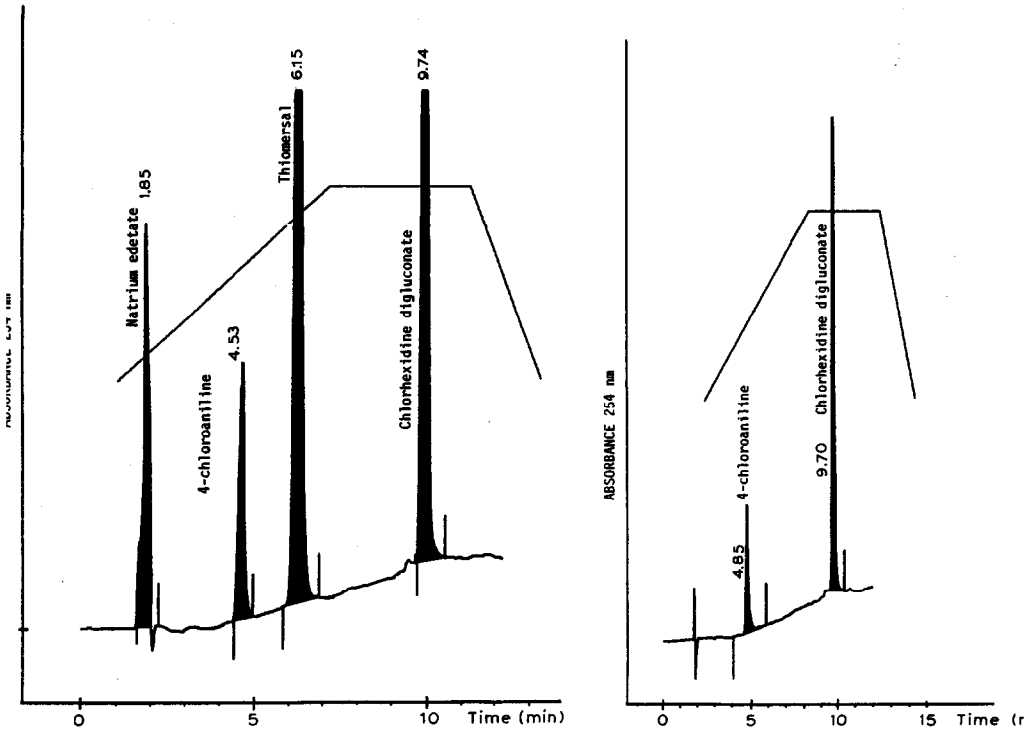

Fig. 1. Chromatogram of EDTA (disodium salt) (10 mg-%), 4-chloroaniline (1.54 mg-%), thiomersal (10 mg-%) and chlorhexidine digluconate (5 mg-%).

Fig. 2. Typical routine chromatogram of 4-chloroaniline and chlorhexidine digluconate solution.

The calibration graph shows a correlation coefficient of 0.9988 in the appropriate range. The linear regression line is $y = 6.1x + 25.04$ where y is the peak area at 254 nm expressed in integration units and x is the amount in nanograms of 4-chloroaniline injected. The detection limit is 11.5 ng of 4-chloroaniline injected.

The reproducibility was determined by injecting 30 μl of a 231 μg per 100 ml solution ten times successively. The relative standard deviation was 1.33%.

Assay of chlorhexidine digluconate

Assays were carried out with chlorhexidine digluconate solutions containing 10, 7.5, 5, 3.75, 2.5, 1.25, 0.625 and 0.312 mg per 100 ml. A 30-μl volume of each solution was injected five times successively.

The calibration graph shows a correlation coefficient of 0.9985 in the appropriate range. The linear regression line is $y = 5.7572x + 96.4058$, where y is the peak area at 254 nm expressed in integration units and x is the amount in nanograms of chlorhexidine digluconate injected. The detection limit is 5.85 ng of chlorhexidine digluconate injected.

The reproducibility was determined by injecting 30 μl of a 5 mg per 100 ml solution ten times successively. The relative standard deviation was 1.58%.

CONCLUSIONS

The method described for determining 4-chloroaniline and chlorhexidine digluconate in the same run is very specific, as no other commonly used eye-drop ingredients (*e.g.*, thiomersal and its degradation products or EDTA) interfere.

The method has been used satisfactorily for over 12 months in our stability laboratory. It was developed mainly for stability studies on pharmaceutical compositions containing chlorhexidine digluconate (such as ophthalmic preparations and contact lens solutions), but it might not be suitable for routine quality control monitoring purposes because it is time consuming.

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