Paper Chromatographic Determination of Chlortetracycline

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Abstract □ Chlortetracycline was successfully separated from its degradation products and from other tetracyclines on a paper chromatogram previously impregnated with a mixture of edetate disodium and urea solution in pH 5.0 McIlvaine buffer. Chlortetracycline separation from its degradation products and from both tetracycline and oxytetracycline was attributed primarily to the complexation of these antibiotics with urea and edetate on the chromatogram. The separated spots were located under UV light, and their R_f values were calculated. Chlortetracycline recovery from the paper chromatogram was satisfactory (98.3 ± 0.002%). The developed method was successfully applied to the determination of chlortetracycline in various dosage forms. In addition, the method was adequate for monitoring chlortetracycline hydrochloride stability in aqueous solutions.

Keyphrases □ Chlortetracycline—paper chromatographic analysis in pharmaceutical preparations □ Chromatography, paper—analysis, chlortetracycline in pharmaceutical preparations □ Antibacterials—chlortetracycline, paper chromatographic analysis in pharmaceutical preparations

Previously (1, 2), paper chromatographic methods were developed for the quantitative determination of oxytetracycline and tetracycline.

Urea was reported to increase the water solubility of chlortetracycline through complex formation (3, 4). The use of a mixture of urea and edetate disodium aqueous solutions as an immobile phase was successfully applied to the paper chromatographic determination of tetracycline in pharmaceutical formulations (2). The objective of the present investigation was to establish a precise quantitative paper chromatographic determination of chlortetracycline in various dosage forms and in the presence of its degradation products.

EXPERIMENTAL

Preparation of Degradation Products—Degradation products of chlortetracycline such as isochlortetracycline, α-aureomycinic acid, β-aureomycinic acid, anhydrochlortetracycline, and dedimethylaminoaureomycinic acid were prepared from chlortetracycline hydrochloride[1] according to reported methods (5). They were separated by partial neutralization of the reaction medium and then filtered through a sintered-glass funnel. The collected precipitate was washed with distilled water until chloride ions disappeared. The products were then dried at 60° over phosphorus pentoxide under vacuum. Melting points, absorption spectra, and paper chromatography data proved their identities.

Impregnating Solution—A 100-ml quantity was prepared by dissolving 5 g of urea and 2 g of edetate disodium in pH 5.0 McIlvaine buffer (6).

Running Solvent—A 90-ml quantity was prepared by mixing 60 ml of chloroform with 30 ml of acetone. The mixture was saturated with solid urea and edetate *disodium*.

Chromatographic Separation of Chlortetracycline—Two series of chromatographic papers[2] were impregnated with the impregnating solution and blotted between sheets of absorbent papers. Then 10 μl of fresh methanolic solutions (1 mg/ml) of each chlortetracycline (international standard)[1], isochlortetracycline, anhydrochlortetracycline, dedimethylaminoaureomycinic acid, α-aureomycinic acid, and β-aureomycinic acid was separately spotted on each chromatogram of the first series while still damp. The second series was separately spotted with 10 μl of the standard fresh methanolic solutions (1 mg/ml) of each chlortetracycline hydrochloride, tetracycline hydrochloride[3], oxytetracycline base[3], and their mixture. The previously published chromatographic procedure (1, 2) was then adopted. The R_f values of the separated spots were calculated (Table I).

Table I—R_f Values and Fluorescence Colors under UV Light of Separated Antibiotics

Product	Fluorescence under UV light	R_f Value
Chlortetracycline	Yellow	0.60
Tetracycline	Yellow	0.24
Oxytetracycline	Yellow	0.10
Isochlortetracycline	Blue	0.34
α-Aureomycinic acid	Violet	0.33
β-Aureomycinic acid	Pale violet	0.13
Anhydrochlortetracycline	Orange	0.99
Dedimethylaminoaureomycinic acid	Pale violet	0.39
Epichlortetracycline	Yellow	0.27 or 0.07

Quantitative Determination of Chlortetracycline—The previously reported horizontal-line technique (1, 2) was applied for the estimation of chlortetracycline in tablets, injections, drops, ointments, suspensions, and solutions. Aliquots of 200 μl of the antibiotic methanolic solution (200 μg) were spotted on the impregnated chromatographic papers. The separated antibiotic zone was eluted by 20 ml of dilute hydrochloric acid (pH 1.8). Chlortetracycline in the acid eluate was determined spectrophotometrically[4] at 368 nm (7).

The procedure previously adopted for preparing samples of oxytetracycline before application to the chromatographic papers (1) was successfully applied for chlortetracycline preparations (Table II).

RESULTS AND DISCUSSION

Paper chromatographic separation of chlortetracycline from its degradation products was found to be most efficient when using papers previously impregnated with a mixture of urea and edetate disodium solution in pH 5.0 McIlvaine buffer. Low R_f values or diffuse spots were observed by separate use of either urea or edetate disodium solutions, respectively. These results were consistent with those previously observed for tetracycline (2).

Table I presents the observed colors of the separated spots under UV light and their R_f values.

This procedure was successfully applied to chlortetracycline separation from either tetracycline or oxytetracycline (Table I). Chlortetracycline was characterized by the highest R_f value, followed in decreasing order by tetracycline and oxytetracycline. Complex formation between tetracycline or oxytetracycline and the components of the impregnating solution was previously proved (1, 2). When chlortetracycline was eluted from the chromatogram using pH 5.0 McIlvaine buffer, it showed a shift of τ_{max} toward the shorter wavelength, thus confirming antibiotic complexation on the chromatogram. The formed complexes of tetracycline and oxytetracycline on the chromatogram were more water soluble than the chlortetracycline complex (Table I). This result was confirmed by the observed relatively higher tendency of the chlortetracycline spot to move with the mobile phase on the chromatogram (high R_f value).

Sharp and complete separation of chlortetracycline from its decomposition products was achieved by applying not more than 20 μg of the antibiotic/spot on the chromatogram. This procedure necessitated the application of 200 μg of the antibiotic as a horizontal line on the chromatographic paper, providing a final concentration of 10 μg/ml in the eluate.

[1] Provided by Lederle Laboratories.
[2] Whatman No. 1.
[3] Provided by the World Health Organization.
[4] Carl Zeiss, Jena.

Table II—Stated and Found Potencies of Chlortetracycline Hydrochloride (I) and Chlortetracycline Base (II) in Different Pharmaceutical Formulations

Dosage Form[a]	Composition	Stated Potency	Found Potency[b], (% *SD*)
Tablets	Collected from market	250 mg/tablet	98.9 (±0.001)
Ointment 1	Collected from market	1% (w/w)	98.0 (±0.010)
Ointment 2	Collected from market	3% (w/w)	99.7 (±0.001)
Ointment 3	I + polyoxyethylene 20 sorbitan (1% w/w) + petrolatum	3% (w/w)	99.3 (±0.002)
Ointment 4	I + absorption ointment base[c]	3% (w/w)	99.3 (±0.002)
Ointment 5	I + wool alcohol ointment[d]	3% (w/w)	99.3 (±0.001)
Injection	Collected from market	500 mg/ampul	95.0 (±0.005)
Drops	Collected from market	5.0 mg/ml	96.0 (±0.005)
Solution 1	I	5.0 mg/ml	100 (±0.001)
Solution 2	I + sodium metabisulfite (0.2% w/v)	5.0 mg/ml	98.0 (±0.002)
Solution 3	I + magnesium chloride hexahydrate (1:1 molar ratio)	5.0 mg/ml	98.0 (±0.001)
Solution 4	I + calcium chloride (1:1 molar ratio)	5.0 mg/ml	100 (±0.006)
Solution 5	I + edetate disodium (1:1 molar ratio)	5.0 mg/ml	98.0 (±0.001)
Solution 6	I + propylene glycol (10% w/w)	5.0 mg/ml	100 (±0.001)
Solution 7	I + polyethylene glycol 400 (10% w/w)	5.0 mg/ml	100 (±0.001)
Solution 8	I + saccharin sodium (10% w/w)	5.0 mg/ml	100 (±0.002)
Solution 9	I + *p*-hydroxybenzoic acid (0.2% w/v)	5.0 mg/ml	98.0 (±0.006)
Solution 10	I + propylparaben (0.2% w/v)	5.0 mg/ml	98.0 (±0.005)
Solution 11	I + sorbic acid (0.5% w/v)	5.0 mg/ml	100 (±0.010)
Suspension 1	II + tragacanth (1.5% w/v)	125 mg/5 ml	100 (±0.002)
Suspension 2	II + tragacanth (0.5% w/v) + sodium alginate (1.5% w/v)	125 mg/5 ml	98.7 (±0.00)
Suspension 3	II + tragacanth (0.5% w/v) + aluminum magnesium silicate[d] (1.2% w/v)	125 mg/5 ml	99.2 (±0.003)
Suspension 4	II + tragacanth (0.5% w/v) + povidone (2% w/v)	125 mg/5 ml	98.7 (±0.001)
Suspension 5	II + tragacanth (0.5% w/v) + carboxymethylcellulose sodium (2% w/v)	125 mg/5 ml	100 (±0.003)
Suspension 6	II + tragacanth (0.5% w/v) + syrup (1% w/v)	125 mg/5 ml	99.2 (±0.001)
Suspension 7	II + tragacanth (0.5% w/v) + glycerin (1% w/v)	125 mg/5 ml	100 (±0.003)
Suspension 8	II + tragacanth (0.5% w/v) + sorbitol (10% w/v)	125 mg/5 ml	98.0 (±0.002)
Suspension 9	II + tragacanth (0.5% w/v) + glycerin (1% w/v) + carmine (1% w/v)	125 mg/5 ml	99.0 (±0.002)
Suspension 10	II + tragacanth (0.5% w/v) + syrup (1% w/v) + carmine (1% w/v) + vitamin B compound[e] (0.2% w/v)	125 mg/5 ml	98.0 (±0.004)

[a] The vehicle used in all solutions and suspensions was purified water. [b] Average of five determinations. [c] USP XVIII, 1970, p. 809. [d] Veegum HV. [e] The British Pharmaceutical Codex, 1973, p. 819.

The recovery of the antibiotic from the chromatogram was found to be satisfactory when using dilute hydrochloric acid (pH 1.8). Chlortetracycline hydrochloride (international standard) was chromatographed to calculate the percent recovery. Nine determinations gave an average recovery of 98.3 ± 0.002%. The slight loss of the antibiotic during elution was greatly compensated for by chromatographing a reference standard for each determination. The absence of degradation spots on rechromatographing the international chlortetracycline standard proved the high stability of this antibiotic during chromatography. The formation of only one defined spot on rechromatographing the acid eluate of international chlortetracycline standard indicated that the antibiotic was not decomposed in this solution for at least 1 hr.

Table II lists the composition and analysis of various chlortetracycline preparations. The found potencies of the tested dosage forms were close to their stated potencies. The additives in the tested formulations did not interfere with the chromatographic determinations of the antibiotic by the presented method. The use of 0.1 *N* HCl to extract the antibiotic from the ointments before application on the chromatogram was unsatisfactory since it interfered with the complexation of chlortetracycline on the chromatogram. The use of distilled water instead of 0.1 *N* HCl as an extracting medium for chlortetracycline hydrochloride ointments overcame this difficulty.

Table III—Stability of Chlortetracycline Hydrochloride Solution (5 mg/ml) in Distilled Water Containing 10% (w/v) Dimethylformamide

Days	Potency, % Microbiological Method	Potency, % Chromatographic[a] Method (%*SD*)
0	100	100 (0.004)
2	95	74.4 (0.001)
3	88	68 (0.04)
4	54.5	50.3 (0.01)
5	51.0	27.3 (0.01)
6	56.0	24.5 (0.01)

[a] Average of five determinations.

Table III shows the potency of chlortetracycline hydrochloride solution (5 mg/ml) in water containing 10% (w/v) dimethylformamide at various time intervals when stored at 40°. The antibiotic contents of the stored solutions were determined by both the prescribed method and the official microbiological procedure (8). Results of the present chromatographic procedure agreed well with the microbiological method for the undecomposed preparation (initial determination). The higher results obtained with the microbiological technique, compared to those obtained with the chromatographic method (Table III), might be attributed to the antimicrobial activity of the decomposition products of chlortetracycline. Table III also indicates that the established chromatographic method is satisfactory for monitoring chlortetracycline stability.

In conclusion, the described method is adequate as either a control assay or a stability-indicating assay for the selective determination of chlortetracycline in dosage forms.

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