Determination of Meclocycline, a Tetracycline Analogue, in Cream Formulations by Liquid Chromatography

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Abstract □ A rapid and sensitive high-performance liquid chromatographic quantitation of meclocycline (I) in a cream formulation is described. The acidified methanolic extract of the sample was diluted with mobile phase and analyzed on a reverse-phase column by using a mobile phase consisting of EDTA buffer (pH 6.6)-tetrahydrofuran (85:15 v/v). The method gave linear, quantitative, and reproducible results with a detection limit of 0.4 ppm for meclocycline.

Keyphrases □ Meclocycline—tetracycline analogue, cream formulations, HPLC □ Cream formulations—meclocycline, tetracycline analogue, HPLC

Meclocycline sulfosalicylate (I), a 7-chloro-6-methylene-5-hydroxy-derivative of tetracycline, is marketed as a topical antibiotic cream formulation[1] for the treatment of acne. Methods for quantitating meclocycline and its impurities have not been reported. However, high-performance liquid chromatographic (HPLC) methods have been shown to be most effective for separating tetracyclines and their degradation products. These HPLC methods have been extensively reviewed (1). The reverse-phase mode has become the most widely used column packing for tetracyclines with a mobile phase at a low pH range (1.2–2.5), which is generally not recommended for bonded silica columns. In an additional method, a microparticulate phenyl column and single-step gradient elution at pH 2.2 are used (2). These types of systems have been found not to be applicable to the separation of meclocycline and its impurities. It has been preferred to have a system with a less acidic mobile phase, not only to prolong the lifetime of the column but to retard the degradation of tetracyclines known to occur at low pH (3). Among the various reverse-phase packings, Vydac TP C_{18} has been found to be very suitable for use with mobile phases in the near neutral region for the separation of tetracyclines and their degradation products (4). By utilizing this column and a mobile phase of tetrahydrofuran in ammonium EDTA buffer at pH 6.6, a baseline separation of meclocycline, and its potential degradation products and impurities in pharmaceutical cream was achieved. In this report is described a rapid, linear, and reproducible method for the quantitation of meclocycline in a cream formulation.

EXPERIMENTAL SECTION

Materials—Meclocycline sulfosalicylate[2] (I) (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy -6- methylene-1,11-dioxo-2-naphthacenecarboxamide, mono(5-sulfosalicylate), was used in all experiments as received. For the cream samples, a 1% w/w formulation of meclocycline sulfosalicylate cream[1] was used. Tetrahydrofuran[3] was HPLC grade; EDTA[4] and all the other chemicals and solvents were analytical reagent grade.

Preparation of Mobile Phase—EDTA (0.6 g) was mixed with 2 mL of methanol and dissolved in 15 mL of concentrated ammonium hydroxide. Approximately 1800 mL of distilled water was added, and the pH was adjusted to precisely 6.6 with glacial acetic acid. The solution was diluted to 2 L with distilled water. The 0.001 M ammonium EDTA buffer solution was filtered through a fluoropore membrane filter (0.5 μm), mixed with tetrahydrofuran in the ratio of 85:15 v/v, and deaerated under vacuum.

Chromatographic System—A liquid chromatograph[5] equipped with an automatic sample injector[6] was used for all HPLC work. The column was packed with octadecylsilane material bonded to microparticulate silica gel (10 μm)[7]. The columns were periodically purged with 95% ethyl alcohol (form III)[8] for optimum resolution. Since the columns were obtained from the manufacturer preserved in denatured alcohol, no column conditioning was necessary. The flow rate was 0.8 mL/min, and a 10-mV strip chart recorder was employed at a chart speed of 0.5 cm/min.

Standard Solution Preparation—A portion (30–50 mg) of the reference standard, meclocycline sulfosalicylate (I) (or 25 mg of USP meclocycline base reference standard) was weighed to the nearest 0.1 mg and dissolved in and diluted to 100 mL with methanol. A 2.0-mL aliquot of the solution described above was diluted to 50.0 mL with mobile phase. The solution was filtered through a Swinney filter with a syringe and used as an external standard. Filters[9] (0.5 μm) were used and stored in methanol prior to use.

Sample Preparation—A portion of the meclocycline sulfosalicylate cream (equivalent to ~8 mg of I or ~5 mg of meclocycline base) was weighed to the nearest 0.1 mg into a 50-mL stoppered centrifuge tube. Equal volumes of methanol (20 mL) and 0.0125 M sulfuric acid (20 mL) were added to the tube. The sample was thoroughly dispersed by a combination of sonication (~60 min) and vortexing (2 min). Inadequate dispersing produced poor recoveries. The dispersed mixture was quantitatively transferred into a 50-mL volumetric flask and diluted to volume with methanol. After centrifugation at ~2000×*g* for 10 min, a 5.0-mL aliquot of the supernatant liquid was diluted to 50.0 mL with the mobile phase. The mixture was filtered through a Swinney filter as described above. The samples should not be stored in the mobile phase solution longer than a day, or loss of meclocycline will be observed.

Analytical Procedure—Chromatograms for all standards and samples were obtained at ambient temperature by injecting 10-μL aliquots at a detector sensitivity of 0.02 AUFS. When the flow rate was 0.8 mL/min, the average retention time of meclocycline was 7 min, although depending on the column condition the retention time can vary between 5.5 and 8.5 min. By small adjustments of tetrahydrofuran in the mobile phase, the retention time may be varied to the desired value. The resolution factors are 3.0 for methacycline and meclocycline and 1.7 for C-4-epimeclocycline and meclocycline, and the tailing factor for meclocycline is 1.0. For quantitation of meclocycline, samples were injected with an automatic sample injector and bracketed with standards in the sequence: STD-1, STD-1, STD-2, STD-2, SAMP-1, SAMP-1, SAMP-2, SAMP-2, STD-1, STD-1, STD-2; STD-2 (where STD is the standard solution and SAMP is the sample solution). A basic program designed for a computer system[10] was used for quantitation of the samples of meclocycline in sequence with the standards as listed above. The percentage of meclocycline base in the cream by weight was calculated by: $(A_{samp} \times W_{std} \times P)/(A_{std} \times W_{samp} \times 50)$. The peak areas and weights, in milligrams, of sample and standard, are A_{samp}, W_{samp}, and A_{std}, W_{std}, respectively. The potency[11] (P) of meclocycline is usually equivalent to not less than 620 μg/mg

[1] Meclan Cream (1%); Ortho Pharmaceutical Corp., Raritan, N.J.
[2] Pfizer Inc., New York, N.Y.
[3] Burdick & Jackson Laboratories, Inc., Muskegon, Mich.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Model 440 244 equipped with a model 6000A pumping system and a model 440 UV detector operated at 340 nm; Waters Associates, Milford, Mass.
[6] WISP 710A; Waters Associates.
[7] Vydac 201 TP reverse phase (25 cm × 3.2 mm i.d.); Separations Group, Hesperia, Calif.
[8] Sargent-Welch Scientific Co. Skokie, Ill.
[9] Fluorophore FHL P04700; Millipore Corp.
[10] HP3356; Hewlett-Packard Co., Palo Alto, Calif.
[11] Meclocycline sulfosalicylate occurs as a monohydrate with a maximum theoretical potency of 669 μg/mg relative to the pure anhydrous base, which has been assigned a potency of 1000 μg/mg.

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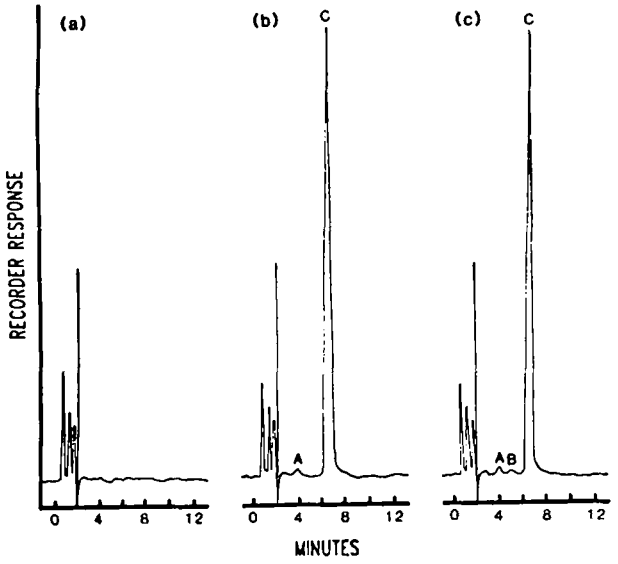

Figure 1—*Typical chromatograms of cream placebo (a), standard I (b), and a 1% cream sample preparation of I (c). Key: (A) methacycline; (B) C-4-epimer of I; (C) I. Conditions: mobile phase, 0.001 M ammonium EDTA buffer (pH 6.6)–tetrahydrofuran (85:15); column, reverse-phase*[7], *25 cm × 3.2 mm i.d.; flow rate, 0.8 mL/min; detector, UV at 340 nm.*

and usually in the area of 665-670 μg/mg[12], and for USP meclocycline base it is 1000 μg/mg.

RESULTS AND DISCUSSION

Typical chromatograms obtained for the cream placebo, standard meclocycline sulfosalicylate (I), and a 1% cream sample preparation of I are shown in Fig. 1. The system gave a baseline separation for meclocycline, methacycline, and the C-4-epimer of meclocycline at ambient temperature. Meclocycline eluted with an average retention time of 7 min. The peak at 4 min corresponded to methacycline, an impurity always present in the raw material but never present at >3% of the total weight. The peak at 5 min was identified to be the C-4-epimer of meclocycline (5). By varying the relative amount of tetrahydrofuran in the mobile phase, the retention times of meclocycline and impurities could easily be shifted. The objective was to keep the retention time of meclocycline in the range of 5.5–8.5 min. The ammonium EDTA buffer in the mobile phase was a crucial ingredient to give well-defined and sharp peaks on HPLC chromatograms. It was also necessary to dilute the sample with the mobile phase in the sample preparation. If only methanol was utilized, leading peaks were observed. The wavelength of 340 nm was selected for maximum sensitivity and for the least amount of interference from UV-absorbing components of cream excipients. The minimum detection for meclocycline was 0.4 ppm at 340 nm.

[12] The potency was determined by a microbiological assay method.

Table I—Recovery of Meclocycline Base from Spiked Cream Placebo [a]

Theoretical Label Claim, %	Percent Recovery[b] Peak Areas	Peak Heights
80	101	100
	97.0	96.2
100	96.0	98.5
	96.8	96.0
120	98.7	98.2
	96.9	97.6
Average	$\bar{X}_6$ = 97.7%	$\bar{X}_6$ = 97.8%
SD	±1.83%	±1.51%
RSD	±1.87%	±1.54%

[a] Representative of 80-120% of theoretical label claim; label claim is 1% w/w meclocycline base. [b] Meclocycline base.

The results of recovery studies performed on cream placebos spiked with I are shown in Table I. The spiking covered the range of 80-120% of the theoretical label claim, which is usually 1% meclocycline base by weight. The recoveries, ranging from 96 to 101%, were comparable whether peak areas or heights were used. The data demonstrate the method to give linear, quantitative, and reproducible results with a minimum of sample handling.

A few other commercially available reverse-phase columns (C_{18}[13,14], ODS[15], and C_8[15]) were tested under the same operating conditions but were found to be not suitable. It seems that meclocycline was bound on these columns and would not elute until purged with methanol. When the solvent system was changed to consist of 1% phosphoric acid–methanol (45:55), the C_{18}[13] reverse-phase column gave a separation of methacycline and meclocycline, but no separation of meclocycline and its C-4-epimer was observed. Since the pH of the latter mobile phase was ~2, meclocycline was assumed to be mainly in the ionic form with a protonated dimethylamino group, which is analogous to other tetracyclines (6). It therefore seems that on reverse-phase columns, the C-4 isomers of meclocycline cannot be separated as ionic species but rather as doubly charged zwitterions, the most likely form of tetracyclines between pH 3.0 and 6.5 reported previously (6).

In conclusion, the described method gives a rapid and reproducible quantitation of meclocycline in the cream formulation of meclocycline sulfosalicylate.

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