Pergamon

0731-7085(93)E0013-D

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 5, pp. 675–692, 1994

0731-7085/94 $7.00 + 0.00

Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases

PETER D. BRYAN* and JAMES T. STEWART

Department of Medicinal Chemistry, College of Pharmacy, The University of Georgia, Athens, GA 30602-2352, USA

Abstract: The LC analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns has been studied. Mobile phases containing acetonitrile–0.02 M sodium perchlorate (pH 2.0) were used. The tetracyclines were detected by their absorbance at 280 nm. The columns included: a polystyrene–divinylbenzene (PS–DVB) column and a polymethacrylate column with octadecyl ligands (PM-C18). Performance of the two columns was compared and applicability of the described methods for compendial use has been evaluated. The tetracyclines investigated include: minocycline, oxytetracycline, tetracycline, demeclocycline, chlortetracycline, methacycline, doxycycline and meclocycline.

Keywords: *LC; pharmaceutical analysis; polymeric columns; minocycline; oxytetracycline; tetracycline; demeclocycline; chlortetracycline; methacycline; doxycycline; meclocycline.*

Introduction

In a previous work [1], methods for the LC separation of selected tetracyclines and their degradation product on polymeric columns were evaluated. The results showed that with acidic mobile phases, the best separations were obtained on a polystyrene–divinylbenzene (PS–DVB) copolymeric column. Good results were also obtained with a column containing a polymethacrylate material with octadecyl ligands (PM-C18). The use of acidic mobile phases allowed for the separation of a wide variety of tetracyclines with ambient column temperatures (25 ± 2°C) and acetonitrile as an organic modifier. A similar method for the bioanalysis of chlortetracycline from bovine milk has also been reported by the authors' laboratory [2].

Most work on the LC analysis of tetracyclines, their degradation products and impurities using polymeric columns has concentrated on the use of viscous organic modifiers and alkaline mobile phases with elevated column temperatures [3–9]. These mobile phases usually required EDTA and the ion-pairing agent tetrabutylammonium hydroxide. A method for the analysis of chlortetracycline using PS–DVB columns with acidic mobile phases has been reported, but the use of 2-methyl-2-propanol in the mobile phase also required the use of elevated column temperatures [10].

In this paper, LC methods for the analysis of selected tetracyclines in bulk drug substance and dosage forms using polymeric columns are described and validated. Similar methods for each individual tetracycline and its corresponding impurities and degradation products, when available, were used. The applicability of these methods for compendial use has been demonstrated for minocycline (MIN), oxytetracycline (OTC), tetracycline (TC), demeclocycline (DMCTC), chlortetracycline (CTC), methacycline (METH), doxycycline (DOX) and meclocycline (MECL).

Experimental

Materials

Reference standards for tetracycline HCl (lot J-1), oxytetracycline (lot I), doxycycline hyclate (lot H), minocycline HCl (lot H), chlortetracycline HCl (lot I and lot I-1), de-

* Author to whom correspondence should be addressed. Present address: Sandoz Pharmaceuticals, 59 Route 10, Bld 401, East Hanover, NJ 07936, USA.

meclocycline HCl (lot G-1) and methacycline HCl (lot G) were obtained from the United States Pharmacopeia (Rockville, MD, USA). Bulk materials for tetracycline HCl (lot 118F-0050), oxytetracycline HCl (lot 67F-0766), doxycycline HCl (lot 49F-0202), minocycline HCl (lot 120H-0266), chlortetracycline HCl (78F-0022), demeclocycline HCl (lot 48F-0182), methacycline HCl (lot 90H-0260) and meclocycline (MECL) sulphosalicylate salt (lot 50H-0682) were obtained from Sigma Chemical Co. (St Louis, MO, USA). 4-Epi-oxytetracycline HCl (EOTC; lot 53383/1), α-apo-oxytetracycline (αAOTC; lot 46693/1), β-apo-oxytetracycline (βAOTC) lot 46694/1), 4-epi-tetracycline HCl (ETC; lot 42832/2), anhydrotetracycline HCl (ATC; lot 47552/1), 4-epi-anhydrotetracycline HCl (EATC; lot 47553/1), 4-epi-chlortetracycline HCl (ECTC; lot 45896/1), isochlortetracycline HCl (isoCTC; lot 45898/1), 4-epi-anhydrochlor-tetracycline HCl (EACTC; lot 458971/1) and anhydrochlortetracycline HCl (ACTC; lot 45900/1) were obtained from Janssen Chimica (Beerse, Belgium).

HPLC grade acetonitrile, perchloric acid (60–62%), concentrated HCl and anhydrous diethyl ether were obtained from J.T. Baker (Phillipsburg, NJ, USA). Sodium perchlorate (99% pure) was obtained from Janssen Chimica and in-house distilled and deionized water was used.

Instrumentation

HPLC analysis was performed using a model 760 pump (Micromeritics, Norcross, GA, USA), a model 710B WISP autosampler (Water Associates, Milford, MA, USA), a model 2550 UV/vis detector set at 280 nm (Varian Analytical Instruments) and a model 4290 integrator (Spectra-Physics, San Jose, CA, USA). The tetracyclines were separated on either a 25 cm × 4.6 mm PLRP-S 100 Å, 5 μm polystyrene–divinylbenzene copolymer column (Polymer Laboratories, Amherst, MA, USA) or a 15 cm × 4.6 mm YMC-Pack PC-02-6 polymer C18, 6 μm, a polymethacrylate polymer with C18 ligands (YMC, Wilmington, NC, USA).

Mobile phases were prepared by mixing various proportions (v/v) of acetonitrile and 0.02 M aqueous sodium perchlorate adjusted to pH 2.0 with perchloric acid. Mobile phases were filtered through a 0.45 μm nylon filter and degassed by sonication before use. Separations were performed at ambient temperature (25 ± 2°C).

Dosage forms were obtained from local pharmacies or from out-of-date supplies at the University of Georgia, College of Pharmacy. The tetracycline dosage forms include: Schein 500 mg capsules (lot 03899c, exp. Oct/92), Schein 250 mg capsules (lot 04963c, exp. Mar/93), Squibb 500 mg Sumycin capsules (lot 6A19969, exp. Jan/91), Squibb 250 mg Steclin capsules (lot unknown, exp. before 1974), Squibb 125 mg Steclin capsules (lot unknown, exp. before 1974). Oxytetracycline dosage forms include: Vedco 100 mg ml^{-1} OTC HCL injectable (lot 9410, exp. Apr/94) and Pfizer Terramycin 250 mg capsules (lot 6404, exp. Nov/90). Doxycycline dosage forms include: Barr Laboratories Doxy-caps 100 mg capsules (lot 1F297AN, exp. Aug/94), Zenith 100 mg tablets (3626-31, exp. Sept/91), Schein 100 mg DOX hyclate capsules (lot 04838, Apr/93). Minocycline dosage forms include: Lederle 100 mg Minocin pellet-filled capsules (lot 296434, exp. Nov/92) and Warner Chilcott 50 mg capsules (lot 1842il, exp. Dec/92). Chlortetracycline dosage form: Lederle Aureomycin 3% ointment (lot 280-677-3s, Apr/95). Demeclocyline dosage form: Lederle 300 mg Declomycin tablets (lot 324-482, exp. Jan/97).

Sample preparation

All standards and dosage form samples were prepared in low actinic volumetric flasks. The tetracycline standard solutions from reference standards and bulk drug samples were prepared by dissolving approximately 1 mg in a low actinic 10 ml volumetric flask and adding 0.02 M sodium perchlorate adjusted to pH 2.0 with perchloric acid, to volume. The standard solutions for the impurities were prepared by dissolving approximately 1 mg in a 100 ml low actinic volumetric flask and adding 0.02 M sodium perchlorate (pH 2.0) to volume. When stored at 4°C, these solutions were stable for approximately 1 working day (8–12 h), with only minimal degradation.

Capsule dosage forms were prepared by placing the entire contents of one capsule in an appropriate volumetric flask to allow for a concentration of approximately 1 mg ml^{-1} and adding 0.02 M sodium perchlorate (pH 2.0) to volume. The flasks were submitted to ultrasonication for 10 min to allow for dispersal and dissolution of the dosage form contents.

Tablets were first crushed with a mortar and pestle, and the contents were transferred to the volumetric flask. The solid dosage form mixtures were filtered through a 25 mm, 0.45 μm nylon filter (Lida Manufacturing, Bensenville, IL, USA) and diluted into an appropriate concentration range with 0.02 M sodium perchlorate pH 2.0. Oxytetracycline injectable dosage forms were directly diluted to the appropriate concentration range with 0.02 M sodium perchlorate (pH 2.0).

CTC ointment was prepared by weighing approximately 250 mg into a separatory funnel. The ointment was dissolved in 30 ml of diethyl ether. CTC was extracted from the organic layer with 0.01 N hydrochloric acid (3 × 25 ml). The sample was transferred to a 100 ml actinic volumetric flask and brought to volume with 0.01 N hydrochloric acid.

Results

Chromatography

Figures 1–7 show the separation of selected tetracyclines on the PS–DVB and the PM-C18 columns, respectively. In each case, the PS–DVB column gave better separation of the tetracyclines from their degradation products and impurities. At 1.0 ml min^{-1}, the back pressure of the PS–DVB column was near the recommended maximum operating pressure (2500 psi), while the PM-C18 column's back pressure was only approximately 1000 psi. For all the tetracyclines and impurities, the elution orders on the PS–DVB and the PM-C18 columns were the same except for TC and diketoCTC. Using the PS–DVB column, TC eluted before diketoCTC and was resolved. With the PM-C18 column, TC eluted slightly after the diketoCTC. When diketoCTC was in great excess, as in the dosage forms, the two peaks were not resolved.

Quantitative measurements of potential degradation products and impurities were performed on as many as were commercially available. In the cases of MIN, DMCTC, DOX, METH and MECL; none of the potential degradation products was commercially

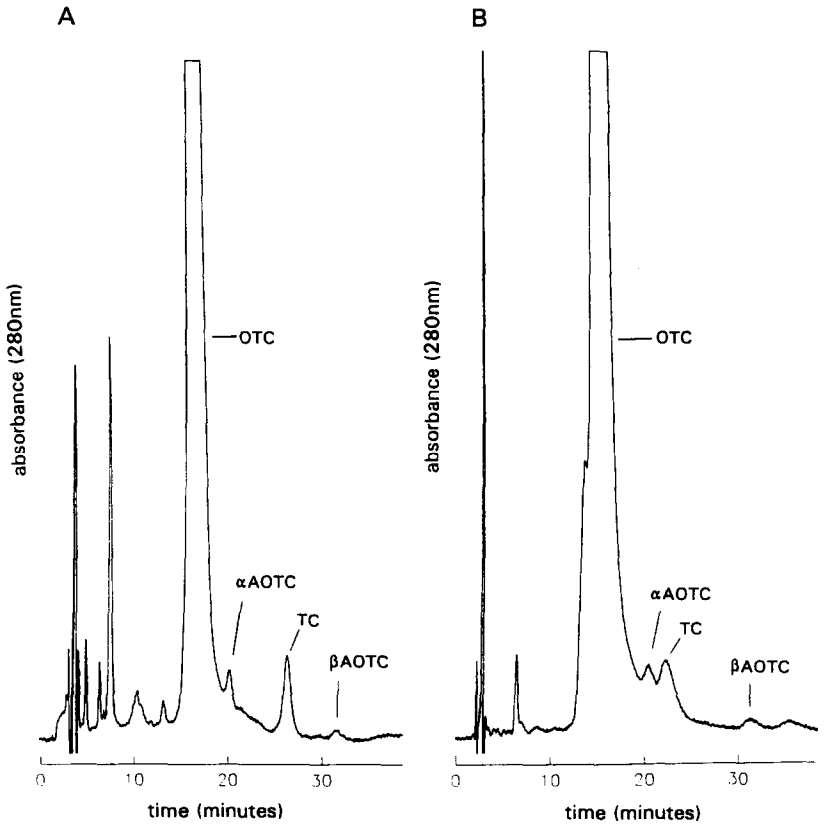

Figure 1
A typical chromatogram from the analysis of an oxytetracycline dosage form (Terramycin 250 mg capsule) using (A) PLRP-S, 25 cm × 4.6 mm i.d.; (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 20:80, v/v), 1.0 ml min^{-1}, 280 nm.

Table 1
Amount of oxytetracycline in dosage forms

	PLRP-S			PM-C18		
	Vedco 100 mg ml^{-1} injectable	Pfizer Terramycin 250 mg capsule	Sigma OTC HCl bulk	Vedco 100 mg ml^{-1} injectable	Pfizer Terramycin 250 mg capsule	Sigma OTC HCl bulk
OTC						
mg ml^{-1}	99.58 ± 1.50	—	—	132.5 ± 2.66	—	—
mg	—	273.8 ± 17.9	3.162 ± 0.047	—	352.8 ± 3.8	3.718 ± 0.056
%RSD	1.51	6.54	1.47	2.01	1.08	1.51
TC						
ng ml^{-1}	43.3 ± 2.5	—	25.0 ± 2.3	<DL	—	<DL
mg	—	1.58 ± 0.22	—	—	1.28 ± 0.17	—
wt%	0.05	0.55	0.04	—	0.36	—
%RSD	5.77	13.7	9.20	—	13.3	—
αAOTC						
ng ml^{-1}	<DL	—	<DL	<DL	—	<DL
mg	—	4.04 ± 0.47	—	—	1.48 ± 0.25	—
wt%	—	1.47	—	—	0.42	—
%RSD	—	11.6	—	—	17.2	—
βAOTC						
ng ml^{-1}	<DL	—	197.1 ± 0.6	<DL	—	<DL
mg	—	5.86 ± 0.49	—	—	0.85 ± 0.20	—
wt%	—	2.04	0.31	—	0.24	—
%RSD	—	8.39	0.30	—	24.1	—
Total impurities						
Area%	0.23	1.23	1.22	1.42	0.63	0.95
wt%	0.05	4.06	0.35	0	1.02	0
%Label	99.6	109.0	100.0	138	141	117
Expiration						
month/year	4/94	11/90	—	4/94	11/90	—

available. For METH and DOX, each were found as impurities in the other; and the amount of METH in DOX and DOX in METH were quantitated. The greatest number of degradation products and impurities were commercially available for OTC, TC and CTC.

Oxytetracycline

The degradation products and impurities available for OTC included EOTC, TC, αAOTC and βAOTC. The mobile phase used for the separation of OTC was acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 20:80, v/v). EOTC could not be separated from OTC in the concentrations found in the dosage forms for the described system because EOTC eluted just after OTC which was in great excess. The three remaining degradation products or impurities were found in the Terramycin 250 mg capsule and were separated by both the PS–DVB and PM-C18 columns (Fig. 1). The peaks obtained on the PS–DVB column were sharper than those obtained on the PM-C18 column.

Table 1 shows the results from the analysis of the OTC dosage forms using the PS–DVB and PM-C18 columns. For the capsule dosage form and the bulk OTC, βAOTC was the impurity found in the highest concentration. In the injectable form, TC was the only commercially available impurity found. Similar results were found using the PM-C18 column except that approximately the same amount of impurities was found and the amount of αAOTC was below the detection limit for that column.

Table 2 shows the analytical figures of merit for the quantitation of OTC using the PS-DVB and the PM-C18 columns. For the PS-DVB column, the regression analysis showed the standard curves to be linear with correlation coefficients >0.999 for OTC, αAOTC and βAOTC. The correlation coefficient for TC in OTC dosage forms was 0.9985 over the range of standards used. Because detection limits for the PM-C18 column were higher, the lowest standard was not detected and only two points could be used for the standard curve.

Standard error for the analysis of OTC using the PS–DVB column is shown in Table 3. The standard error for the PS–DVB column was generally lower than with the PM-C18 column. Using the PS–DVB column, the standard error for the high, middle and low standards were + 0.84, −3.20 and +4.57, respectively, while for the PM-C18 column, the standard errors were +1.55, −5.93 and +8.48, respectively.

Tetracycline

The degradation products and impurities commercially available for TC include ETC, CTC, EATC and ATC. The mobile phase used for the analysis of TC in dosage forms was acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v). All of the available de-

Table 2
Analytical figures of merit for the quantitation of oxytetracycline, tetracycline and chlortetracycline (PLRP-S)

Analyte	Regression equation	r^2	Standard range (μg ml^{-1})	Detection limit (μg ml^{-1})	Detection limit (ng)	Detection limit (% Area)
OTC						
OTC (area)	$y = 1531.0x - 20614$	0.9992	20.99–94.81	15*	750*	—
TC (area)	$y = 1804.2x + 153.82$	0.9985	0.0520–2.079	0.025*	1.2*	0.03
TC (ht)	$y = 40.251x + 3.9890$	0.9985	0.0520–2.079	0.025*	1.2*	0.03
αAOTC (ht)	$y = 28.110x - 0.6343$	0.9995	0.0509–0.5090	0.050	2.5	0.05
βAOTC (ht)	$y = 11.774x + 0.3491$	0.9999	0.0531–0.5306	0.050	2.5	0.05
TC						
TC (area)	$y = 95{,}531x + 32{,}647$	0.9999	10.10–90.89	0.08*	4*	—
ETC (area)	$y = 78{,}197x + 78{,}165$	0.9997	0.2222–43.83	0.20	10	0.2
CTC (ht)	$y = 10.237x - 0.400$	1.0000	0.1251–12.51	0.05*	2.5*	0.06
EATC (ht)	$y = 36.326x - 0.875$	0.9960	0.0467–0.1868	0.04	2	0.04
ATC (ht)	$y = 38.519x - 0.052$	0.9986	0.0429–0.1714	0.04	2	0.04
CTC						
CTC (area)	$y = 2418.7x - 4817.4$	0.9993	11.15–112.5	3*	150*	—
ECTC (area)	$y = 2201.0x - 6.457$	1.0000	0.0548–94.16	0.05	2.5	0.04
TC (area)	$y = 5968.0x + 243.5$	1.0000	0.0529–5.289	0.02*	1.0*	0.02
EACTC (ht)	$y = 59.624x + 0.6002$	0.9999	0.0402–2.011	0.04	2.0	0.04
ACTC (ht)	$y = 61.678x + 0.8801$	1.0000	0.0444–2.274	0.04	2.0	0.04

* Estimated detection limit (signal/noise = 3).

Table 3
Standard error of the analysis of oxytetracycline, tetracycline and chlortetracycline

	Actual	Calculated	%Error	Actual	Calculated	%Error	Actual	Calculated	%Error
OTC									
OTC	94.81	95.81	+0.84	54.45	52.71	−3.20	20.99	21.95	+4.57
TC(ar)	2.079	2.074	−0.24	0.2079	0.2715	+23.4	0.1040	0.1011	−27.9
TC(ht)	2.079	2.074	−0.24	0.2079	0.2728	+23.8	0.1040	0.1015	−24.4
αAOTC	0.5090	0.5099	+0.18	0.1010	0.0937	−7.23	0.0509	0.0581	+12.4
βAOTC	0.5306	0.5309	+0.06	0.1061	0.1037	−2.26	0.0531	0.0553	+3.80
TC									
TC	90.89	90.36	−0.58	50.50	51.44	+1.86	10.10	9.78	−3.17
ETC	43.83	50.80	+13.7	2.207	3.290	+32.8	0.5555	0.3883	−30.1
CTC	12.51	12.51	0	1.251	1.211	−3.28	0.1251	0.1612	+21.8
EATC	0.1868	0.1893	+9.50	0.0934	0.0860	−7.92	0.0467	0.0516	+1.32
ATC	0.1714	0.1701	−6.06	0.0857	0.0896	+4.35	0.0429	0.0403	−7.58
CTC									
CTC	112.54	113.52	+0.87	56.27	54.04	−3.96	11.25	12.47	+10.8
ECTC	5.289	5.293	+0.08	0.5289	0.5162	−2.40	0.0529	0.0648	+22.5
TC	94.16	94.15	−0.01	5.483	5.687	+3.72	0.5483	0.3990	−27.2
EACTC	2.011	2.008	−0.15	0.4021	0.4163	+3.53	0.0421	0.0305	−27.55
ACTC	2.274	2.272	−0.09	0.4437	0.4559	+2.75	0.0444	0.0344	−22.52

gradation products and impurities were well separated from the TC peak using the described chromatographic conditions (Fig. 2).

The results from the analysis of TC dosage forms are shown in Table 4. For the three dosage forms which were still in-date or recently out-of-date, all of the degradation products except ETC were not detected. The detection limits obtained with the PM-C18 column were higher than with the PS–DVB column. Two TC dosage forms were analysed which have expiration dates before 1974. These dosage forms contained all of the degradation products and impurities available. The 125 mg capsule was especially degraded, containing only 64% of the labelled amount of TC.

The regression equations and limits of detection for TC, ETC, CTC, EATC and ATC are shown in Table 2. The correlation coefficients for the regression equation for TC, ETC and CTC were >0.999, showing the standard curve to be linear within the range of standards used.

The standard errors for the analysis of TC and its degradation products are shown in Table 3. Relatively high standard errors were seen for ETC which eluted just prior to the TC peak.

Chlortetracycline

The degradation products and impurities commercially available for CTC are ECTC, TC, EACTC, ACTC and isoCTC. The mobile phase used for the analysis of CTC was acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 30:70, v/v). All the available degradation products and impurities were separated from the CTC and the unknown peaks, except for isoCTC, which coeluted with an impurity (Fig. 3).

Results from the analysis of CTC dosage forms, bulk sustance and a USP reference standard material are shown in Table 5. Of the available degradation products/impurities, only ECTC and TC were detected. Using the PM-C18 column, TC could not be detected in the samples because of its coelution with a

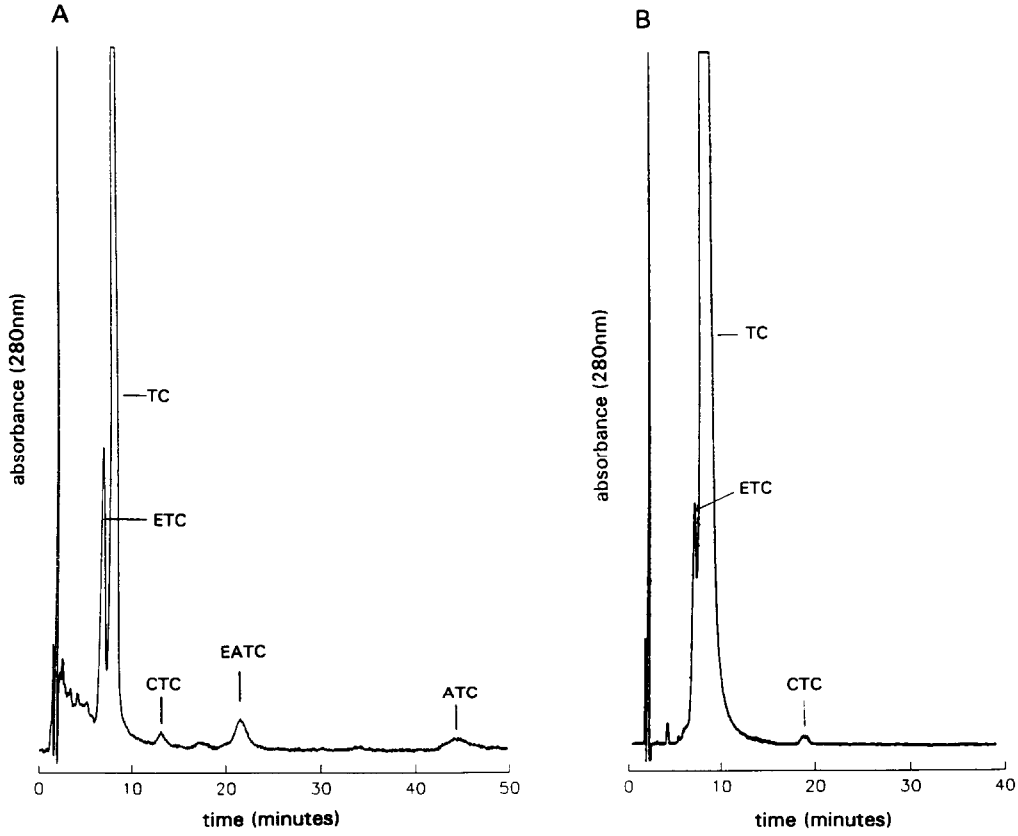

Figure 2
A typical chromatogram from the analysis of a tetracycline dosage form (Steclin 250 mg capsule) using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile-sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v), 1.0 ml min^{-1}, 280 nm.

Table 4
Amount of tetracycline in dosage forms (PLRP-S)

	Schein 500 mg capsules*	Schein 250 mg capsules*	Squibb Sumycin 500 mg capsules*	Squibb Steclin 250 mg capsules†	Squibb Steclin 125 mg capsules†	Sigma TC HCl bulk*
TC						
mg	525.5	264.3	518.0	252.3	92.8	2.975
SD	4.7	4.9	6.6	1.4	1.6	0.045
%RSD	0.89	1.87	1.27	—	—	1.51
ETC						
mg	<DL	<DL	<DL	5.20	11.73	0.0481
SD	—	—	—	0.36	0.66	0.0008
wt%	—	—	—	2.08	12.64	1.62
%RSD	—	—	—	—	—	1.61
CTC						
mg	<DL	<DL	<DL	0.855	2.32	0.0254
SD	—	—	—	0.125	0.27	0.0014
wt%	—	—	—	0.34	2.50	0.85
%RSD	—	—	—	—	—	5.55
EATC						
mg	<DL	<DL	<DL	0.51	0.78	<DL
SD	—	—	—	0.08	0.09	—
wt%	—	—	—	0.02	0.84	—
%RSD	—	—	—	—	—	—
ATC						
mg	<DL	<DL	<DL	0.69	1.03	0.0027
SD	—	—	—	0.08	0.19	0.0006
wt%	—	—	—	0.03	1.11	0.09
%RSD	—	—	—	—	—	22.6
Total impurities						
Area%	3.28	4.05	4.95	3.68	63.7	2.97
wt%	0	0	0	2.47	17.1	2.56
%Label	105.9	104.9	103.6	100.9	74.2	98.9
Expiration month/year	10/92	3/93	1/91	<1974	<1966	—

*$n = 3$.
†$n = 2$.

Table 5
Amount of chlortetracycline in dosage forms

	PLRP-S			PM-C18		
	Lederle Aureomycin 3% ointment	USP reference standard (lot I)	Sigma CTC HCl bulk	Lederle Aureomycin 3% ointment	USP reference standard (lot I)	Sigma CTC HCl bulk
CTC						
mg	8.214	10.76	8.297	7.911	10.26	8.119
SD	0.093	0.10	0.103	0.082	0.13	0.163
%RSD	1.13	0.93	1.24	1.22	1.22	2.01
ECTC						
mg	0.1880	0.0754	2.948	0.4750	0.5005	2.013
SD	0.0090	0.0021	0.086	0.0430	0.0356	0.393
wt%	2.29	0.70	3.55	6.16	0.66	3.14
%RSD	4.79	2.78	2.91	9.05	7.11	19.54
TC						
mg	<DL	<DL	0.0892	ND*	ND*	ND*
SD	—	—	0.0880	—	—	—
wt%	—	—	10.11	—	—	—
%RSD	—	—	10.49	—	—	—
EACTC						
mg	<DL	<DL	<DL	<DL	<DL	<DL
SD	—	—	—	—	—	—
wt%	—	—	—	—	—	—
%RSD	—	—	—	—	—	—
ACTC						
mg	<DL	<DL	<DL	<DL	<DL	<DL
SD	—	—	—	—	—	—
wt%	—	—	—	—	—	—
%RSD	—	—	—	—	—	—
Total impurities						
Area%†	55.9	1.96	33.9	24.0	2.60	7.98
wt%	2.29	0.70	13.7	6.16	0.66	3.14
% Label	98.5	100.1	78.9	94.9	95.4	77.2
Expiration month/year	4/95	—	—	4/95	—	—

*Tetracycline was not resolved from diketochlortetracycline using the PM-C18 column.
†Excludes the diketo tautomer of chlortetracycline.

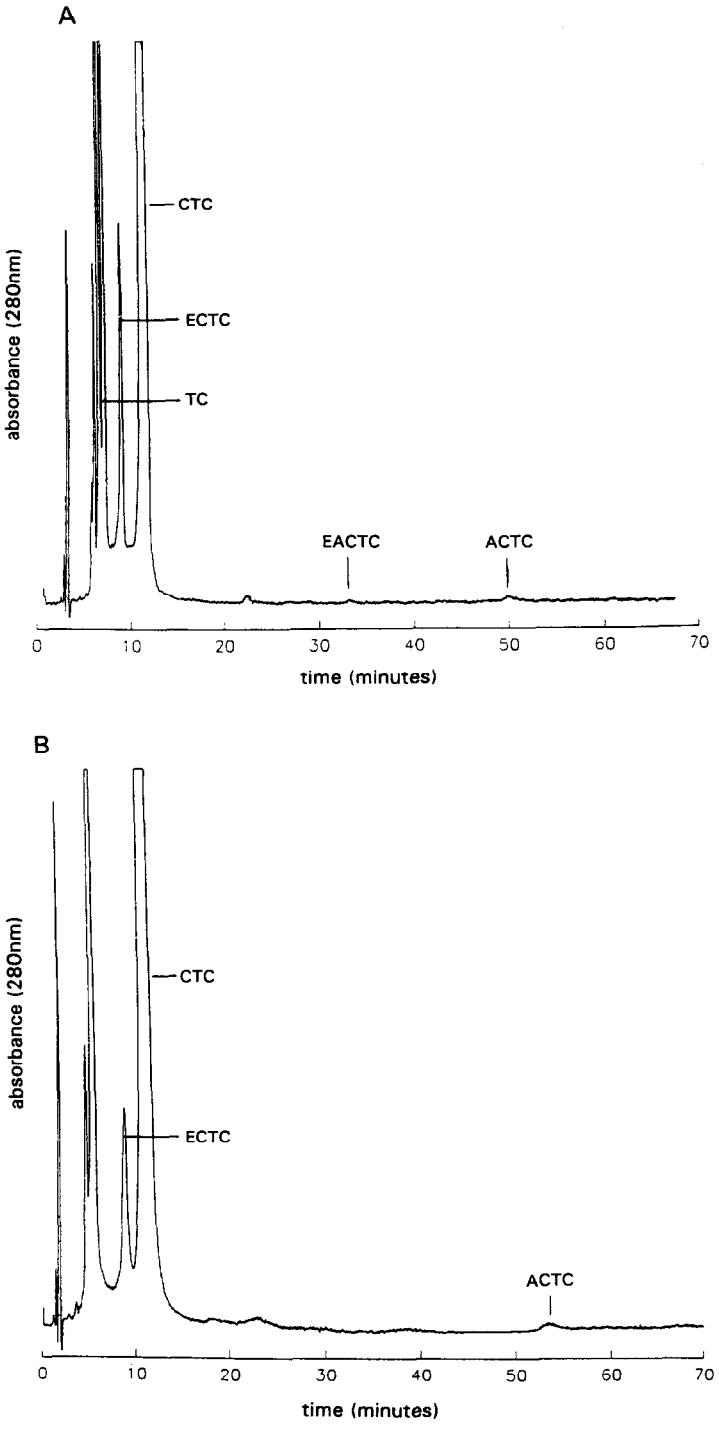

Figure 3
A typical chromatogram from the analysis of a chlortetracycline dosage form (Aureomycin 3% ointment) using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 30:70, v/v), 1.0 ml min^{-1}, 280 nm.

peak which is probably the diketo tautomer of CTC. For all the samples analysed, EACTC and ACTC were below their detection limits. The bulk CTC HCl contained small amounts of ECTC and contained a significant amount of TC (10.5%). Small amounts of ECTC were also found in all of the CTC samples analysed including the USP reference standard.

The regression equations and limits of detection using the PS–DVB column for CTC,

ECTC, EACTC and ACTC are shown in Table 2. Correlation coefficients for the regression equations were all >0.999.

Standard errors for the analysis of CTC and its degradation products were shown in Table 3. Errors for the high standards were <1% for CTC and its degradation products.

Doxycycline

METH and OTC were the impurities commercially available for the analysis of DOX. None of its degradation products was available. The mobile phase used for the analysis of DOX was acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v). Representative chromatograms from a dosage form using the two columns are shown in Fig. 4. METH was separated from DOX on the PS–DVB column, but were not separated on the PM-C18 column. Resolution between a number of the unknown peaks was also lost on the PM-C18 column. When using the described chromatographic conditions, a relatively large impurity peak was observed at 9 or 13 min in the chromatogram for the PM-C18 or PS–DVB column, respectively. The UV spectrum of the peak was similar to that obtained for the diketo tautomer of CTC [11]. This peak was observed in all the DOX samples. Also the same ratio of diketoDOX to DOX was observed for all DOX samples.

Table 6 shows the results obtained from the analysis of DOX dosage forms. The synthetic impurity METH was present in all of the dosage forms. None of the samples analysed contained detectable amounts of OTC. Tables 7 and 8 show the regression equation/detection limits and standard errors, respectively, for the analysis of DOX.

Methacycline

OTC and DOX are potential impurities available for the analysis of METH. DOX was the only commercially available impurity found from the analysis of METH bulk drug. Chromatograms from the analysis of METH

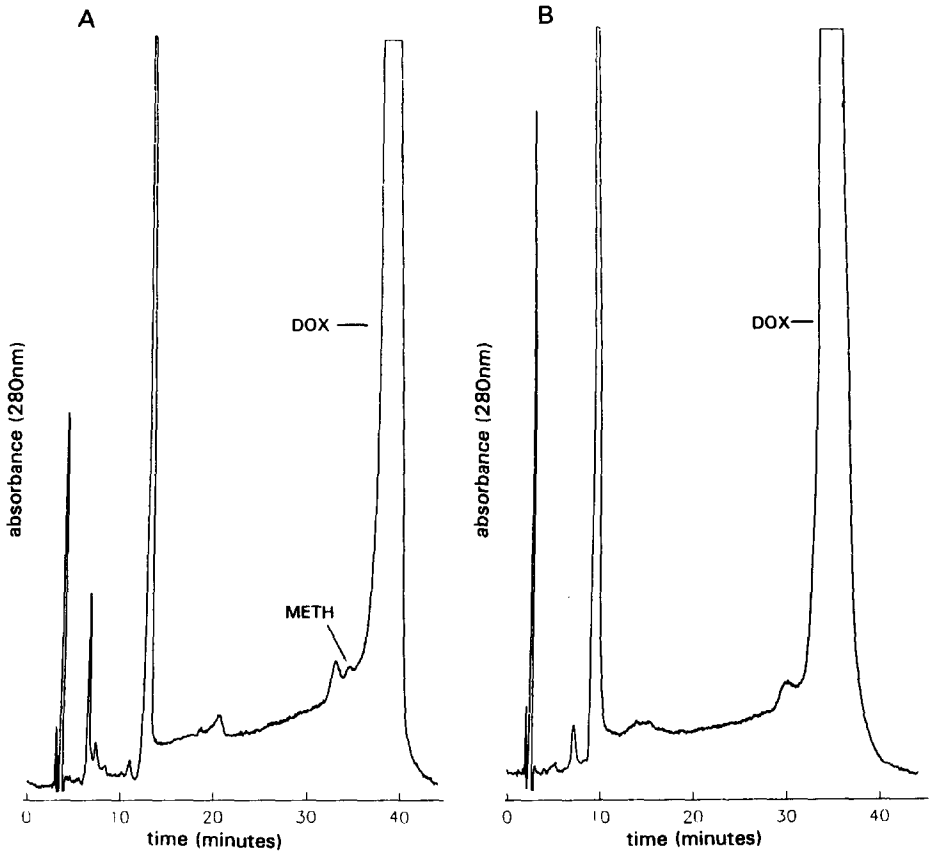

Figure 4
A typical chromatogram from the analysis of a doxycycline dosage form (Zenith 100 mg tablets) using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v), 1.0 ml min^{-1}, 280 nm.

Table 6
Amount of doxycycline in dosage forms

	PLRP-S				PM-C18			
	Barr Doxy-caps 100 mg capsule	Schein 100 mg capsule	Zenith 100 mg tablet	Sigma DOX HCl bulk	Barr Doxy-caps 100 mg capsule	Schein 100 mg capsule	Zenith 100 mg tablet	Sigma DOX HCl bulk
DOX								
mg	109.5 ± 2.31	110.8 ± 1.47	106.3 ± 1.96	2.652 ± 0.014	110.2 ± 1.85	111.2 ± 1.57	107.6 ± 1.83	3.055 ± 0.037
%RSD	2.11	1.33	1.84	0.54	1.68	1.41	1.70	1.20
METH								
mg	5.49 ± 0.55	1.21 ± 0.09	1.90 ± 0.19	0.0785 ± 0.0053	ND*	ND*	ND*	ND*
wt%	5.02	1.09	1.79	2.96	—	—	—	—
%RSD	10.1	7.34	9.89	6.74	—	—	—	—
OTC								
mg	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
wt%	—	—	—	—	—	—	—	—
%RSD	—	—	—	—	—	—	—	—
Total impurities								
Area%†	9.69	2.67	9.23	4.73	1.83	2.07	3.02	2.56
wt%	5.02	1.09	1.79	2.96	0	0	0	0
%Label	110	111	106	85.1	110	111	108	93.7
Expiration								
month/year	8/94	9/91	9/91	—	8/94	9/91	9/91	—

*METH was not resolved from DOX using the PM-C18 column.
†Excludes the diketo tautomer of doxycycline.

Table 7
Analytical figures of merit for the quantitation of doxycycline, methacycline, demeclocycline, minocycline and meclocycline

Analyte	Regression equation	r^2	Standard range ($\mu g\ ml^{-1}$)	Detection limit*		
				($\mu g\ ml^{-1}$)	(ng)	(% Area)
PLRP-S						
DOX	$y = 740.73x - 4673.5$	0.9999	7.487–74.87	2.0	100	—
METH	$y = 1818.6x + 289.21$	0.9999	11.07–110.65	0.5	25	—
DMCTC	$y = 1299.7x - 601.35$	1.0000	8.233–82.33	1.0	50	—
MIN	$y = 1519.9x + 3015.8$	0.9994	11.25–112.53	1.0	50	—
MECL	$y = 750.84x + 2899.4$	0.9990	7.785–77.85	1.0	50	—
PM-C18						
DOX	$y = 525.76x - 3993.8$	0.9987	7.608–76.08	8.0	400	—
METH	$y = 1104.0x - 1422.6$	1.0000	11.07–110.65	2.0	100	—
DMCTC	$y = 742.12x - 1086.5$	0.9999	8.233–82.33	2.0	100	—
MIN	$y = 114.3x - 3678.3$	0.9997	11.25–112.53	4.0	200	—
MECL	$y = 478.94x + 3647.2$	0.9995	7.785–77.85	2.0	100	—

*Estimated detection limit (signal/noise = 3/1).

Table 8
Standard error of the analysis of doxycycline, methacycline, minocycline, demeclocycline and meclocycline

	Actual	Calculated	%Error	Actual	Calculated	%Error	Actual	Calculated	%Error
PLRP-S									
DOX	103.96	104.15	+0.18	51.98	51.55	−0.83	10.40	10.63	+2.21
METH	110.65	110.26	−0.35	55.35	56.23	+1.59	11.07	10.58	−4.43
DMCTC	82.33	82.37	+0.05	41.17	41.07	−0.24	8.23	8.28	+0.61
MIN	112.53	113.46	+0.83	56.27	54.18	−3.71	11.25	12.41	+10.3
MECL	77.85	77.05	−1.03	38.93	40.73	+4.62	7.785	6.784	−12.9
PM-C18									
DOX	76.08	76.99	+1.20	38.04	35.98	−5.42	7.61	7.79	+2.37
METH	110.65	110.77	+0.11	55.35	55.08	−0.49	11.07	11.22	+1.36
DMCTC	82.33	82.49	+0.19	41.17	40.80	−0.90	8.23	8.43	+2.43
MIN	112.53	113.11	+0.52	56.27	54.82	−2.58	11.25	12.04	−7.02
MECL	77.85	77.31	−0.69	38.93	40.15	+3.13	7.785	7.107	−8.71

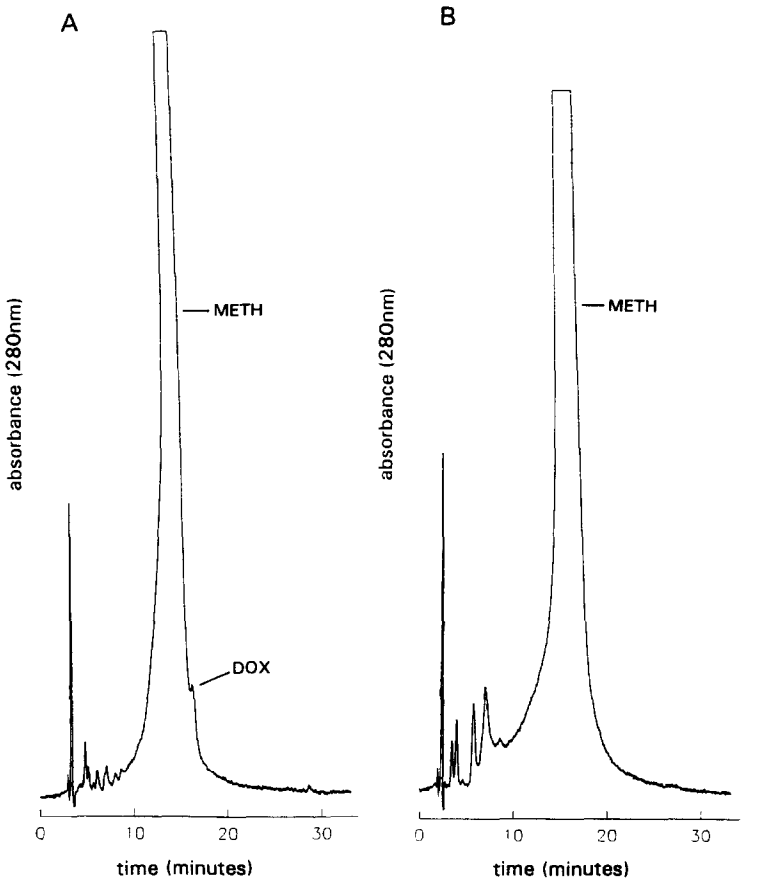

Figure 5
A typical chromatogram from the analysis of a methacycline HCl bulk drug substance using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 30:70, v/v), 1.0 ml min^{-1}, 280 nm.

are shown in Fig. 5. The mobile phase used for the analysis of METH was acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 30:70, v/v). As in the case of DOX, only the PS–DVB column was able to separate METH from its DOX impurity. Even when the mobile phase for the analysis of METH contained 30% acetonitrile, DOX was separated from METH. Using the PS-DVB column, DOX was detected in the METH bulk drug at the 0.02% level and OTC was below the detection limit. Results from the analysis of METH bulk drug are shown in Table 9. Tables 7 and 8 show the regression equations/limits of detection and standard errors, respectively, for the analysis of METH bulk drug.

Minocycline, demeclocycline and meclocycline

For the analysis of MIN dosage forms, mobile phases containing acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 20:80, v/v) were

Table 9
Amount of methacycline in bulk drug substance

	Sigma METH HCl bulk (PLRP-S)	Sigma METH HCl bulk (PM-C18)
METH		
mg	4.227 ± 0.033	4.197 ± 0.058
%RSD	0.77	1.39
DOX		
mg	0.0008 ± 0.0001	ND*
wt%	0.02	—
%RSD	12.7	—
OTC		
mg	<DL	<DL
wt%	—	—
%RSD	—	—
Total impurities		
area%	0.16	0
wt%	0.02	0
%Label	103.8	103.0
Expiration month/year	—	—

* DOX was not resolved from METH using the PM-C18 column.

used. For DMCTC, mobile phases containing acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v) were used. Chromatograms for the analysis of MIN and DMCTC dosage forms are shown in Figs 6 and 7, respectively. For MECL sulphosalicylate bulk drug samples, mobile phases containing acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 35:65, v/v) were used. Using the PLRP-S and the PM-C18 columns, the retention times for MECL were 35 and 36 min, respectively. Results from the analysis of MIN, MECL and DMCTC are also shown in Table 10. The regression equation/detection limits and standard error for the analysis of MIN, DMCTC and MECL are shown in Tables 7 and 8, respectively. Reference standard materials were not available for MECL, therefore purity could only be expressed in terms of % area (Table 10).

Discussion

Chromatography

The general chromatographic conditions for the separation of tetracyclines, their degradation products and impurities have been presented in a previous work [1]. In this work, the mobile phases contained smaller amounts of organic modifier than previously described. The mobile phases were chosen to allow for the separation of the many degradation products and impurities which potentially could be formed by fermentation, semi-synthesis and degradation of the tetracyclines investigated.

In every case, the PS–DVB column had the higher efficiency and greater resolution than the PM-C18 column, as previously shown [1]. Because of the higher back pressure observed with the PS–DVB column, the PM-C18 has the distinct advantage that higher flow rates could be used. Using the PM-C18 column, flow rates as high as 2.0 ml min^{-1} were used without exceeding the upper pressure limit recommended by the manufacturer. This would allow for shorter retention times and higher sample throughput.

Oxytetracycline

As shown by the data, OTC, TC, αAOTC and βAOTC were well separated and the

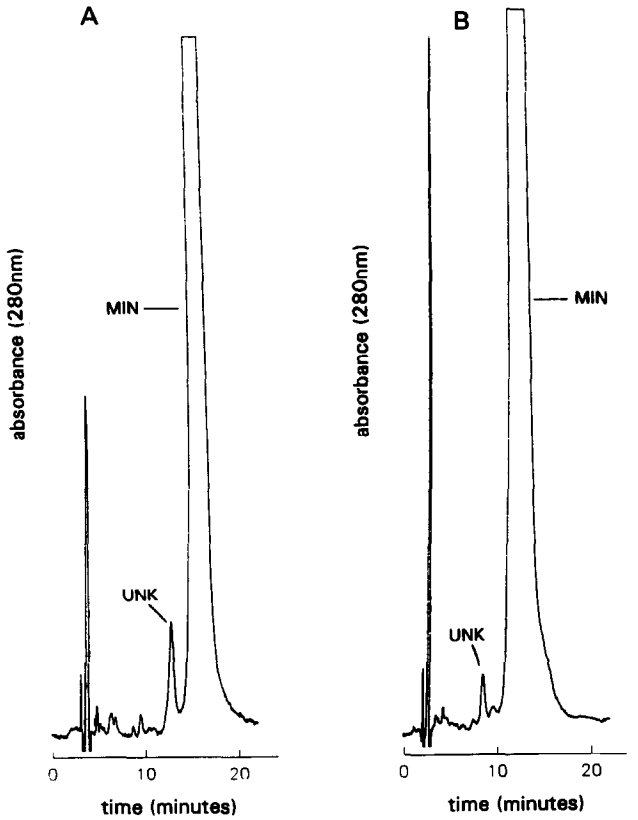

Figure 6
A typical chromatogram from the analysis of a minocycline dosage form (Warner Chilcott 50 mg capsule) using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 20:80, v/v), 1.0 ml min^{-1}, 280 nm.

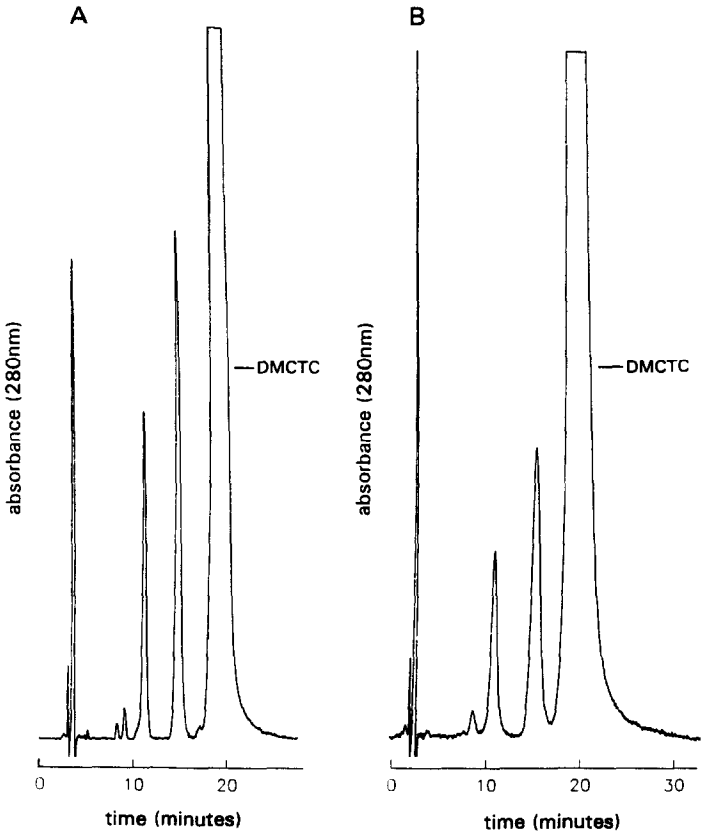

Figure 7
A typical chromatogram from the analysis of a demeclocycline dosage form (Declomycin 300 mg tablet) using (A) PLRP-S, 25 cm × 4.6 mm i.d., (B) PM-C18, 15 cm × 4.6 mm i.d. Acetonitrile–sodium perchlorate (pH 2.0, 0.02 M, 25:75, v/v), 1.0 ml min^{-1}, 280 nm.

Table 10
Amount of demeclocycline, minocycline and meclocycline in dosage forms

	mg	%RSD	Total impurities (area%)	%Label	Expiration date (month/year)
PLRP-S					
Minocycline					
Warner Chilcott (50 mg capsule)	48.82 ± 1.32	2.71	0.47	97.6	12/92
Lederle Minocin (100 mg capsule)	125.2 ± 3.03	2.42	1.99	125	11/92
Sigma MIN HCl bulk	2.789 ± 0.006	0.21	1.68	87.7	—
Demeclocycline					
Lederle Declomycin (300 mg tablet)	306.1 ± 1.4	0.46	11.6	102	1/97
Sigma DMCTC HCl bulk	2.385 ± 0.014	0.59	22.0	99.9	—
Methacycline					
Sigma METH sulphosalicylate	—	1.43	5.64	—	—
PM-C18					
Minocycline					
Warner Chilcott (50 mg capsule)	51.87 ± 0.88	1.69	0.87	103.8	12/92
Lederle Minocin (100 mg capsule)	99.54 ± 1.42	1.43	0.40	100.5	11/92
Sigma MIN HCl bulk	2.818 ± 0.071	2.51	0.46	88.6	—
Demeclocycline					
Lederle Declomycin (300 mg tablet)	301.6 ± 0.1	0.03	11.6	102	1/97
Sigma DMCTC HCl bulk	2.348 ± 0.015	0.64	28.6	98.5	—
Methacycline					
Sigma METH sulphosalicylate	—	0.60	3.53	—	—

regression equation was linear over the standard range used. Standard errors for the analysis of OTC, TC, αAOTC and βAOTC were low for the upper range of standards but became larger at the lower standards. Standard errors for the analysis of αAOTC in OTC were relatively large at the intermediate and low concentrations because the αAOTC peak eluted on the tail of the OTC peak. Larger errors would be expected for the quantitation of a peak which elutes on the tail or shoulder of another peak, especially in the case where the tailing peak was in large excess. EOTC could not be separated from OTC in the described system because it eluted just after the OTC peak, which was in great excess. Because the EOTC and OTC peaks were not separated, the amount of EOTC in the samples could not be analysed. In equal concentrations in the μg ml^{-1} range, EOTC and OTC were slightly separated on the PS–DVB column. If the quantitation of both OTC and EOTC are of particular interest, an HPLC system which allows for the elution of EOTC prior to OTC may be more appropriate [8].

Tetracycline

For the analysis of tetracycline, both the PS–DVB and PM-C18 columns separated all of the available impurities. ETC and TC were not well separated on the PM-C18 column. Standard error and detection limits were higher for the PM-C18 column than the PS–DVB column because of the lower resolution between ETC and TC. The lower efficiency of the PM-C18 column caused broader peaks and higher detection limits.

It is interesting to note that the 250 mg Steclin capsules which may be 20 years old were still within the limits allowed by the USP. The labelled amount of TC was within the stated active ingredient range of 90–125%. The samples would have passed the limit test requirements because the capsules contained <3% EATC. The 125 mg Steclin capsule also was under the 3% limit for EATC, although the amount of TC found in the dosage form was out of the allowed active ingredient range. Even though the scope of these results are limited, it suggests that the 2% limit for EATC in bulk TC substances and 3% limit in dosage forms established by the USP may be too high. This limit may have been set when analytical procedures were only able to detect EATC at the 2% level in a TC sample. The detection limit for the described method is at least 50 times lower than the limits established by the USP.

Chlortetracycline

Three CTC samples were analysed using the described methods. Using the PS–DVB column, both the USP reference material (lot-I) and the CTC bulk were within 1% of the labelled amount. The bulk CTC HCl was labelled by the supplier as 80% by HPLC. The method described herein found 79% CTC and 10% TC in this bulk sample. By microbiological assay, the amount of CTC was claimed to be 97% by the supplier. These results show that the microbiological assays can significantly overestimate the amount of labelled tetracycline. In this case, the microbiological assay even overestimated the total amount of tetracycline activity (CTC plus TC) in the bulk drug substance.

The PM-C18 column would not be an appropriate column for the analysis of CTC in dosage form or bulk drug, because the impurity TC coeluted with the diketo tautomer of CTC.

Diketo CTC was not found in the reference standard material, but was rapidly formed when CTC was dissolved in solution. A mobile phase pH of 2.0 was chosen to minimize formation of the C4 epimer and the diketo tautomer of CTC. The CTC tautomer comes to equilibrium with CTC after approximately 15 min. In order to allow for accurate results using this method, samples and standard solutions were allowed to stand at room temperature for approximately 20 min prior to HPLC analysis. This allowed the ratio of diketo CTC to CTC to become constant. The same procedure should also be followed for DOX and DMCTC. These two tetracyclines are also believed to form a corresponding diketo tautomer in solution. The diketo tautomers are separated by the methods described herein. When diode array detection was used, the suspected diketo tautomers had a UV spectrum which suggests their identity to be the corresponding diketo tautomer.

As discussed above for TC, the detection limits for EACTC are well below the limit established for the epianhydrotetracyclines by USP monographs. The methods described above would allow for a lowering of the limit test amounts of epianhydrotetracyclines in dosage forms and bulk drug substance.

Unlike the other tetracyclines, CTC is quite unstable under alkaline conditions. Only acidic mobile phases can be used for the analysis of CTC.

Doxycycline

Similar results were obtained using the PS–DVB and PM-C18 columns for the analysis of DOX. The primary difference between the results from the two columns was the inability of the PM-C18 column to resolve METH from DOX. As mentioned above for CTC, DOX samples and standard solutions should be allowed to stand at ambient temperature for approximately 20 min to allow the suspected diketo tautomer of DOX to come to equilibrium with DOX.

Methacycline

For the analysis of METH, only a bulk drug sample was obtained. Only small amounts of DOX (0.02%) were detected in the METH bulk drug. The method described was linear and showed little error over the range of standards used for the analysis of METH bulk drug.

Minocycline, demeclocycline and meclocycline

Standards for the degradation products of MIN, DMCTC and MECL, were not commercially available and the ability of the PS–DVB and PM-C18 columns to separate degradation products could not be evaluated. For MECL, no reference standards were available, therefore quantitation of the bulk drug substance was not possible. Standard curves for these three tetracyclines were linear within the range of standards used; standard errors were low.

Conclusions

Liquid chromatographic methods for the analysis of selected tetracyclines using acidic mobile phases and polymeric columns in bulk drug substance and dosage forms have been developed for OTC, TC, CTC, DOX, MIN, DMCTC, METH and MECL. Analysis of commercially available impurities and degradation products have been included where possible. These methods have been shown to be selective, sensitive, accurate and precise.

The methods described show particular advantages over other methods described for the analysis of tetracyclines by allowing for ambient column temperatures to be used. Also, the amount of CTC in a TC dosage form can be analysed in a single method. The described procedures also allow for the analysis of the anhydro degradation products of TC or CTC to be performed under isocratic conditions. In addition to the anhydro tetracyclines, the methods separate many of the impurities which elute before the tetracycline of interest.

It is recommended that the HPLC methods described herein be considered as suitable replacements for existing official USP monograph procedure for tetracyclines.

References

[1] P.B. Bryan and J.T. Stewart, *J. Pharm. Biomed. Anal.* **11**, 971–976.
[2] P.D. Bryan, K.R. Hawkins, J.T. Stewart and A.C. Capomacchia, *Biomed. Chromatogr.* **6**, 305–310 (1992).
[3] J. Hoogmartens, N.H. Khan, H. Vanderhaeghe, A.L. Van Der Leeden, M. Oosterbaan, G.L. Veld-Tulp, W. Plugge, C. Van Der Vlies, D. Mialanne, R. Melamed and J.H.McB. Miller, *J. Pharm. Biomed. Anal.* **7**, 601–610 (1989).
[4] W. Naidong, J. Thuranira, K. Vermeulen, E. Roets and J. Hoogmartens, *J. Liq. Chromatogr.* **15**, 2529–2549 (1992).
[5] N.H. Khan, P. Wera, E. Roets and J. Hoogmartens, *J. Liq. Chromatogr.* **13**, 1351–1374 (1990).
[6] K. Wolfs, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.* **385**, 444–447 (1986).
[7] W. Naidong, E. Roets and J. Hoogmartens, *J. Pharm. Biomed. Anal.* **7**, 1691–1703 (1989).
[8] N.H. Khan, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.* **405**, 229–245 (1987).
[9] W. Naidong, K. Verresen, E. Roets and J. Hoogmartens, *J. Chromatogr.* **586**, 61–66 (1991).
[10] W. Naidong, J. De Beer, X. Marcelis, P. Derese, J.H.McB. Miller and J. Hoogmartens, *J. Pharm. Biomed. Anal.* **10**, 199–204 (1992).
[11] W. Naidong, E. Roets, R. Busson and J. Hoogmartens, *J. Pharm. Biomed. Anal.* **8**, 881–889 (1990).

[Received for review 1 June 1993; revised manuscript received 25 October 1993]