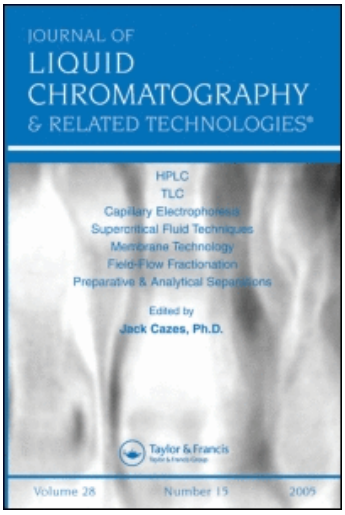


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Analysis of Minocycline by Liquid Chromatography on Poly(styrene-divinylbenzene)

Weng Naidong[ab]; J. Thuranira[a]; K. Vermeulen[a]; E. Roets[a]; J. Hoogmartens[a]
[a] Katholieke Universiteit Leuven, Instituut voor Farmaceutische Wetenschappen, Laboratorium voor Farmaceutische Chemie, Leuven, Belgium [b] Analytical Services Department, Harris Laboratories Inc., Lincoln, NE, U.S.A.

To cite this Article Naidong, Weng , Thuranira, J. , Vermeulen, K. , Roets, E. and Hoogmartens, J.(1992) 'Quantitative Analysis of Minocycline by Liquid Chromatography on Poly(styrene-divinylbenzene)', Journal of Liquid Chromatography & Related Technologies, 15: 14, 2529 — 2549
To link to this Article: DOI: 10.1080/10826079208017200
URL: http://dx.doi.org/10.1080/10826079208017200

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(14), 2529-2549 (1992)

QUANTITATIVE ANALYSIS OF MINOCYCLINE BY LIQUID CHROMATOGRAPHY ON POLY(STYRENE-DIVINYLBENZENE)

WENG NAIDONG*, J. THURANIRA, K. VERMEULEN, E. ROETS, AND J. HOOGMARTENS

Katholieke Universiteit Leuven
Instituut voor Farmaceutische Wetenschappen
Laboratorium voor Farmaceutische Chemie
Van Evenstraat 4
B-3000 Leuven, Belgium

ABSTRACT

An isocratic liquid chromatographic method has been developed for assay and purity control of minocycline. All the potential impurities are well separated from the main component and from each other. The stationary phase is poly(styrene-divinylbenzene) (PSDVB), PLRP-S, 250 mm x 4.6 mm, which is heated at 60 °C. The mobile phase is 2-methyl-2-propanol (x g/100 ml) - 0.2 M potassium phosphate buffer pH 10.5 (10.0 ml) - 0.02 M tetrabutylammonium sulphate pH 10.5 (10.0 ml) - 0.01 M sodium edetate pH 10.5 (10.0 ml) - water (up to 100 ml). The flow rate is 1.0 ml/min and UV detection is performed at 254 nm. The total analysis time does not exceed 30 min. Official standards were compared and a number of commercial bulk samples and specialties were analysed. 4-Epiminocycline, 6-deoxy-6-demethyltetracycline and 7-monodemethylminocycline are the main impurities. 7-Didemethylminocycline, 9-minocycline and some impurities of unknown identity also can be present.

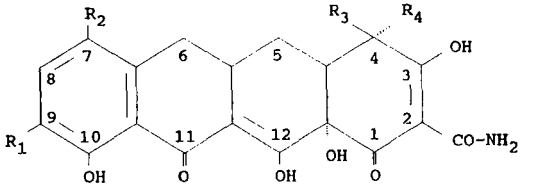
[1]Present address : Harris Laboratories Inc., Analytical Services Department, 624 Peach Street, P.O. Box 80837, Lincoln, NE 68501, U.S.A.

INTRODUCTION

Minocycline (MC) is a tetracycline antibiotic obtained by semi-synthesis from demeclocycline (DMCTC) [1]. 6-Deoxy-6-demethyltetracycline (6-DODMTC), 7-didemethylminocycline (7-DDMMC) and 7-monodemethylminocycline (7-MDMMC) are intermediates. 9-Minocycline (9-MC) is a synthetic side product. In solution and upon storage MC is prone to epimerization, resulting in the formation of 4-epiminocycline (EMC). Structures of MC and its related substances are shown in Fig. 1. MC is used as the monohydrochloride (MC.HCl).

The United States Pharmacopeia (USP) XXII prescribes a liquid chromatographic (LC) method for the assay and purity control of MC, using a silica-based reversed-phase C8 column with a mobile phase consisting of dimethylformamide (DMF) and aqueous solutions of ammonium oxalate and sodium edetate between pH 6.2 and 6.5 [2]. A very similar method is also prescribed by the British Pharmacopoeia (BP) 1988 but here the pH of the mobile phase has to be adjusted in the range pH 6.2 - 7.0 [3]. The same method was recently taken up in the French Pharmacopoeia (Ph. Fr.), but here the pH has to be adjusted to 7.0 exactly [4]. An evaluation of these methods is described elsewhere [5].

In this paper, an isocratic method is described, using poly(styrene-divinylbenzene) (PSDVB) as the stationary phase. It enables the complete separation of MC from its related substances 7-DDMMC, 7-MDMMC, EMC, 6-DODMTC and 9-MC. The method is based upon LC methods previously elaborated in this laboratory for the analysis of doxycycline [6-8], tetracycline [9,10], oxytetracycline [11,12], DMCTC [13] and metacycline [14]. The method has been used to compare official standards and to analyse a number of commercial samples.



	R_1	R_2	R_3	R_4
Minocycline (MC)	H	$N(CH_3)_2$	H	$N(CH_3)_2$
6-Deoxy-6-demethyl-tetracycline (6-DODMTC)	H	H	H	$N(CH_3)_2$
7-Didemethylminocycline (7-DDMMC)	H	NH_2	H	$N(CH_3)_2$
7-Monodemethylminocycline (7-MDMMC)	H	$NHCH_3$	H	$N(CH_3)_2$
9-Minocycline (9-MC)	$N(CH_3)_2$	H	H	$N(CH_3)_2$
4-Epiminocycline (EMC)	H	$N(CH_3)_2$	$N(CH_3)_2$	H

Figure 1. Chemical structure of minocycline and related substances.

EXPERIMENTAL

Reference Substances and Samples

The United States Pharmacopeia Reference Standard (USP-RS) (Lot H, 857 μg/mg), the British Pharmacopoeia Chemical Reference Substance (BP-CRS) (Lot 1425, 92.5 % as the hydrochloride) and a Lederle Laboratories (Pearl River, NY, USA) Working Standard (L-WS) (Lot 13035B-134-100, 857.6 μg/mg) were available. A house standard of MC.HCl (91.0 % m/m) was prepared in the laboratory. Reference substances of EMC.HCl (content not specified), 6-DODMTC.HCl (99.3 % m/m), 7-DDMMC.HCl (content not specified) and 9-MC (92.1 % m/m) were kindly donated by

Lederle-Cyanamid (Louvain-la-Neuve, Belgium). Small quantities of 7-MDMMC were obtained by methylation of 7-DDMMC.HCl and isolated by a thin-layer chromatographic method previously described for identification of tetracyclines [15].

Bulk samples of MC.HCl were obtained from different plants of manufacturer A and from manufacturers B and C. Samples of capsules, tablets and powder for injection produced by manufacturer A were obtained from the Belgian market.

Solvents and Reagents

2-Methyl-2-propanol from Janssen Chimica (Beerse, Belgium) was distilled before use. Tetrabutylammonium hydrogen sulphate was from Janssen Chimica. Other reagents were of pro analysi quality (Merck, Darmstadt, Germany). Water was freshly distilled twice from glass apparatus.

LC Equipment and Operating Conditions

The LC apparatus consisted of a L-6200 pump (Merck-Hitachi, Darmstadt, Germany), a Marathon autosampler equipped with a 20 µl loop (Spark Holland, Emmen, The Netherlands), a Merck-Hitachi L-4000 UV detector set at 254 nm and an integrator model 3393 A (Hewlett-Packard, Avondale, PA, USA). The poly(styrene-divinylbenzene) (PSDVB) PLRP-S 8 µm, 100 Å, 300 Å and 1000 Å (Polymer Labs, Church Stretton, Shorpshire, UK) and PRP-1 8 µm, 10 µm (Hamilton, Reno, NV, USA) were packed in 250 mm x 4.6 mm I.D. columns following a described method [16]. The column was maintained at 60 °C in an oven. The flow rate was 1.0 ml/min. The backpressure was between 30 and

70 bar, depending on the brand and porosity of the stationary phase. The mobile phase finally used with a PLRP-S 100 Å column consisted of 2-methyl-2-propanol (7.0 g) - 0.2 M potassium phosphate buffer pH 10.5 (10.0 ml) - 0.02 M tetrabutylammonium (TBA) sulphate pH 10.5 (10.0 ml) - 0.01 M sodium edetate (EDTA) pH 10.5 (10.0 ml) - water (up to 100.0 ml). During preparation of the 0.02 M TBA or 0.01 M EDTA solutions, the pH was adjusted to 10.5 with sodium hydroxide solution. The mobile phase was degassed by sonication. Each evening the pump was washed with mixture of water and methanol (50:50) but columns were never washed.

Sample Preparation and Stability

About 25.0 mg of MC.HCl bulk sample were accurately weighed, dissolved in 0.01 N sodium hydroxide containing 0.1 % m/v of sodium sulfite and diluted to 25.0 ml with the same solvent. For capsules and tablets, a sample equivalent to about 25.0 mg of MC.HCl was diluted to 25.0 ml with the above-mentioned solvent. The mixture was sonicated for 5 min at room temperature and then centrifuged at 2500 *g* for 5 min. The supernatant liquid was filtered through a membrane filter with 1.5 μm pores. When the solution was stored at 6 °C in the autosampler, the EMC content increased from 0.30 % to 0.32 % in a period of about 36 h. The surface area for the MC peak or for related substances other than EMC did not change and no additional peaks were formed. In 0.01 N HCl as the solvent, the EMC content increased from 0.9 % to 2.0 % in a period of 24 h.

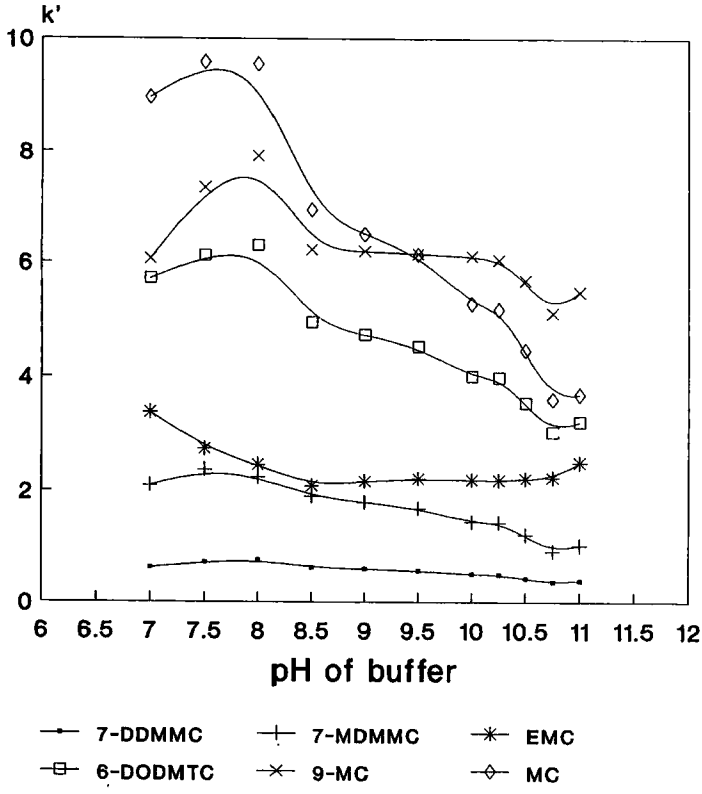


Figure 2. Influence of the pH of the mobile phase on the separation of minocycline and its related substances. Stationary phase : PLRP-S, 8 μm, 100 Å. Mobile phase : 2-methyl-2-propanol (8.0 g) - 0.02 M phosphate buffer of pH indicated (10.0 ml) - 0.02 M TBA (10.0 ml) - 0.01 M EDTA (10.0 ml) - water (up to 100.0 ml). The pH of the TBA and EDTA solutions was brought to the pH indicated with sodium hydroxide solution. See experimental for other conditions.

RESULTS AND DISCUSSION

Development of a Chromatographic Method

Experience obtained with LC analysis on PSDVB of other tetracyclines [6-14] was used for the development of the method. As for the other tetracyclines, 2-methyl-2-propanol was chosen as the organic modifier. The influence of the pH of the mobile phase on the separation is shown in Fig. 2. At pH 10.5, all the potential impurities are well separated from MC and from each other. It was also observed that the peak symmetry and the efficiency were much better at higher pH. In experiments at pH 7.5 and 10.5, where for the latter the organic modifier was reduced from 8.0 to 7.0 % m/v to obtain the same retention time for MC, the symmetry for the MC peak was 1.05 (pH 10.5) against 1.22 (pH 7.5) and the efficiency was 2500 (pH 10.5) against 1270 (pH 7.5). The stability of the PSDVB stationary phase is very good, even at extreme pH values [17]. Fig. 3 shows the influence of the concentration of 2-methyl-2-propanol on the separation. A mobile phase with 7 % m/v of 2-methyl-2-propanol was chosen for further study. Fig. 4 shows the influence of the concentration of TBA. The presence of TBA was necessary to obtain good separation of 7-MDMMC and EMC. Since the amount was not critical 10 % v/v 0.02 M TBA was finally chosen as for other tetracyclines. The influence of the phosphate buffer concentration is minor. In order to keep the total salt concentration at a minimum level and to have enough buffer capacity, a content of 10 % v/v was used in all the experiments. The presence of EDTA in the mobile phase was necessary, otherwise the MC peak would be distorted. An amount of 10 % v/v 0.01 M EDTA was used in all the experiments.

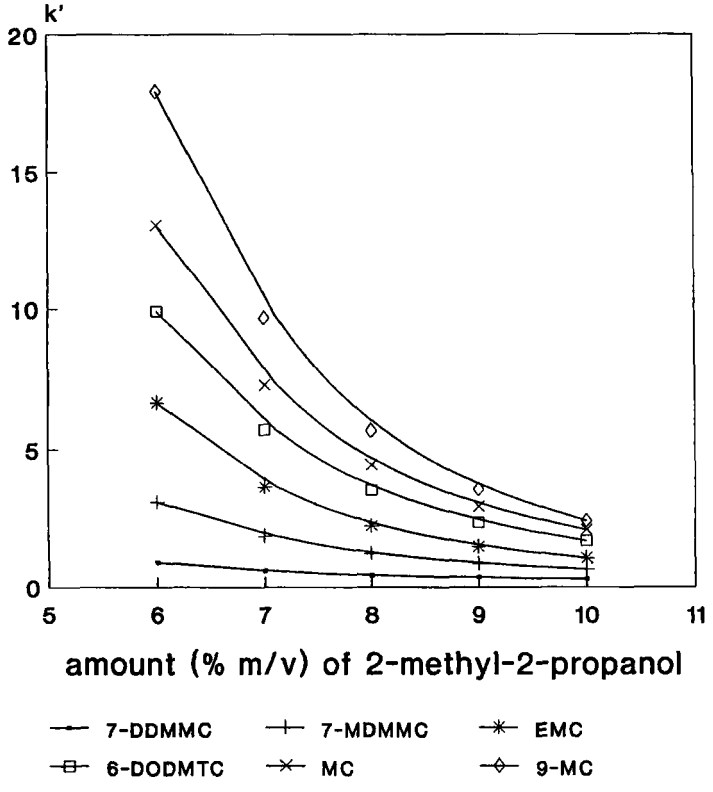


Figure 3. Influence of the concentration of 2-methyl-2-propanol in the mobile phase on the separation of minocycline and its related substances.
Stationary phase : PLRP-S, 8 µm, 100 Å. Mobile phase : 2-methyl-2-propanol (x g) - 0.2 M phosphate buffer pH 10.5 (10.0 ml) - 0.02 M TBA pH 10.5 (10.0 ml) - 0.01 M EDTA pH 10.5 (10.0 ml) - water (up to 100.0 ml). See experimental for other conditions.

A column temperature of 60 °C was maintained throughout the study. This temperature was also found suitable for analysis of other tetracyclines [6-14]. The influence of the temperature on the stability of MC during analysis was checked by repeated analysis of MC.HCl house standard at 50 °C and 60 °C. The surface

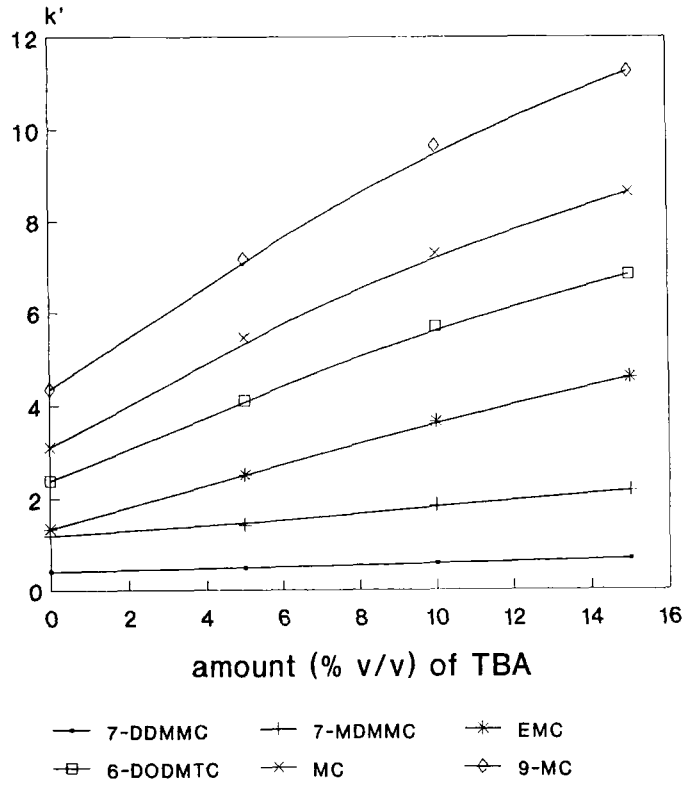


Figure 4. Influence of the concentration of tetrabutylammonium in the mobile phase on the separation of minocycline and its related substances.
Stationary phase : PLRP-S, 8 µm, 100 Å. Mobile phase : 2-methyl-2-propanol (7.0 g) - 0.2 M phosphate buffer pH 10.5 (10.0 ml) -0.02 M TBA pH 10.5 (x ml) - 0.01 M EDTA pH 10.5 (10.0 ml) - water (up to 100.0 ml). See experimental for other conditions.

area for MC was identical at both temperatures. The content of EMC, the most easily formed degradation product of MC, was not found to increase with temperature. A better symmetry and a higher plate number were obtained at 60 °C.

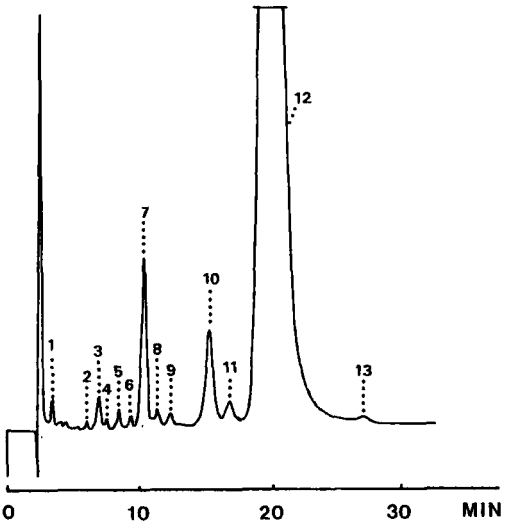


Figure 5. Chromatogram of an old sample of minocycline hydrochloride.
Stationary phase : PLRP-S, 8 μm, 100 Å, 250 mm x 4.6 mm ID. Mobile phase : 2-methyl-2-propanol (7.0 g) - 0.2 M phosphate buffer pH 10.5 (10.0 ml) - 0.02 M TBA pH 10.5 (10.0 ml) - 0.01 M EDTA pH 10.5 (10.0 ml) - water (up to 100.0 ml). Flow rate : 1.0 ml/min. Detection : UV at 254 nm. Temperature : 60 °C.
S = solvent peak, UNK = substance of unknown identity, 1 = 7-DDMMC (0.08 %), 2 = UNK (0.02 %), 3 = 7-MDMMC (0.20 %), 4 = UNK (0.02 %), 5 = UNK (0.12 %), 6 = UNK (0.06 %), 7 = EMC (1.30 %), 8 = UNK (0.11 %), 9 = UNK (0.12 %), 10 = 6-DODMTC (1.16 %), 11 = UNK (0.27 %), 12 = MC, 13 = 9-MC (0.06 %).

Fig. 5 shows a chromatogram of an old sample obtained on PLRP-S, 8 μm, 100 Å. It clearly demonstrates the selectivity towards impurities of known or unknown identity. DMCTC, the starting material for the semi-synthesis of MC was eluted with the same retention time as UNK 5. Demethyltetracycline (DMTC), the intermediate of the conversion of DMCTC to 6-DODMTC, was eluted close after 7-DDMMC. Since DMTC was never detected in samples,

it was concluded that UNK 5 most probably was not DMCTC. Recent samples contain less of the unknown impurities. A good separation was also obtained on other PSDVB materials available on the market. Characteristics of the different stationary phases examined are shown in Table 1. The separation pattern was similar on all the columns. PSDVB with wider pores had no particular advantages, in contradistinction with observations previously made for LC of chlortetracycline or erythromycin [16,18], where better separations were obtained on the wider pore materials. Even a column which was in use for more than five years still showed a selectivity comparable with a new column, but the plate number was lower. With silica-based reversed-phase materials, it is often observed that selectivity depends much on the brand of stationary phase but also on column age and history. All further analyses in this study were performed on a column packed with PLRP-S, 8 μm, 100 Å.

Calibration Curves, Repeatability and Limits of Quantitation

Calibration curves were constructed with MC.HCl house standard and with reference substances for 6-DODMTC.HCl and 9-MC. The following relationships were found, where y = peak area, x = mass injected, in micrograms, corrected for the content of the sample, r = correlation coefficient, $S_{y,x}$ = standard error of estimate, R = range of injected mass examined, l = concentration levels examined, n = number of analyses at each concentration level; MC.HCl : y = 8227x - 5941, r = 0.9987, $S_{y,x}$ = 1302, R = 14-24 μg, l = 8, n = 1; MC.HCl : y = 7955x - 8, r = 0.9999, $S_{y,x}$ = 89, R = 0.06-2.3 μg, l = 5, n = 2; 6-DODMTC.HCl : y = 7827x + 17, r = 0.9999,

TABLE 1
Characteristics of Stationary Phases

Column	Column age (year)	Amount (% m/v) of 2-methyl-2-propanol in mobile phase	Plate number per column MC	Peak symmetry MC	Resolution EMC-MC	Capacity factor k'					
						7-DDMMC	7-MDMMC	EMC	6-DODMTC	MC	9-MC
PLRP-S, 100 Å, 8 μm	new	7.0	2500	1.05	7.11	0.58	1.84	3.66	5.70	7.29	9.67
PLRP-S, 300 Å, 8 μm	1.5	6.5	2650	1.33	7.46	0.47	1.53	3.00	4.70	6.12	8.14
PLRP-S, 1000 Å, 8 μm	1.5	5.0	2650	1.28	5.74	0.36	1.21	2.57	3.78	4.82	6.92
PRP-1, 10 μm	6	6.8	1600	1.32	6.24	0.81	2.10	3.49	5.97	7.65	9.75
PRP-1, 7-9 μm	0.5	7.0	2350	1.17	6.99	0.64	2.02	4.04	6.27	7.99	10.58

See Fig. 5 for chromatographic conditions other than amount of organic modifier.

TABLE 2
Composition of Minocycline Hydrochloride Standards

	House standard	USP-RS 857 μg/mg	BP-CRS 92.5 %	L-WS 857.6 μg/mg
Number of solutions	6	6	6	6
Number of analyses	3	3	3	3
Total number of analyses	18	18	18	18
Number of days	3	3	3	3
7-DDMMC	0.02 (37)	≤0.01 (LOQ)	≤0.01 (LOQ)	≤0.01 (LOQ)
7-MDMMC	0.13 (12)	0.05 (20)	0.06 (14)	0.06 (12)
UNK 6[a]	0.15 (21)	0.06 (29)	0.06 (16)	0.05 (13)
EMC	1.14 (4)	0.38 (8)	0.44 (14)	0.37 (8)
UNK 9[a]	0.07 (27)	0.07 (38)	0.06 (30)	0.06 (21)
6-DODMTC	0.42 (12)	0.07 (49)	0.06 (56)	0.06 (57)
MC.HCl	91.0 (0.8)	91.9 (0.8)	91.6 (0.7)	91.7 (0.6)
MC		85.1	84.8	84.9
9-MC	0.06 (21)	<0.03 (LOQ)	<0.03 (LOQ)	<0.03 (LOQ)
Subtotal	93.0	92.5	92.3	92.3
Water determined (Karl Fischer)	7.0	ND	ND	ND
(n;RSD)	(5;0.8)			
Water declared		7.43[b]	NA	NA
Total	100.0	99.9		

Values in percent m/m. LC results expressed in terms of MC.HCl. n = number of analyses. RSD (%) values are given in parentheses. ND = not determined owing to the limited amount of sample. NA = not available. UNK = unknown. LOQ = limit of quantitation. [a] The number corresponds to the peak number in fig. 5. [b] Ref. 19.

$S_{y,x}$ = 49, R = 0.02-1.0 μg, l = 5, n = 2; 9-MC.HCl : y = 8213x - 50, r = 0.9998, $S_{y,x}$ = 89, R = 0.02-1.0 μg, l = 5, n = 2.

The calibration curves were not used to calculate the content of the sample but only to check the linearity and to compare the absorbance of the related substances with that of MC. The calculations for the content of the main component were based on the results obtained for the MC.HCl house standard (high or low concentration) in each series of analyses. Since reference substances for 7-DDMMC, 7-MDMMC, EMC, 6-DODMTC and 9-MC are not easily available and their exact contents were not verified, the content of all the impurites was expressed as MC.HCl. From the results reported above it is seen that the absorbance of the impurities, as far as could be checked, is very similar to that of MC.HCl. The limit of quantitation (L.O.Q.) was 0.01 % m/m for 7-DDMMC and 7-MDMMC, 0.02 % m/m for EMC and 6-DODMTC and 0.03 % m/m for 9-MC. At these levels the relative standard deviation (RSD) was < 50 %. The house standard was analysed 35 times over a period of 3 days, the RSD for MC was 0.9 %.

Comparison of Minocycline Hydrochloride Standards

The MC.HCl house standard was titrated with perchloric acid in non-aqueous conditions, using acetic acid (HOAc) or acetic anhydride (Ac_2O) as the solvent. In HOAc the mean expressed as MC.HCl was 94.3 % (n = 11, RSD = 0.5 %). In Ac_2O this mean was 92.4 % (n = 11, RSD = 1.0 %). In the first series only free base was titrated, in the second the chloride ions were also titrated. These results indicate that 1.8 % m/m of the minocycline is not present as the hydrochloride, while 90.5 % is present as the hydrochloride. A total of 5 Karl Fischer titrations gave a mean of 7.0 % m/m water (RSD = 0.8 %). Since the

total mass was well explained by the titrations, the total content of dry mass in the house standard was accepted to be 100.0 - 7.0 = 93.0 % m/m and this value was further corrected by means of chromatography. The total amount of chromatographic impurities corresponds to 2.0 % m/m expressed as MC.HCl. Therefore, the MC.HCl house standard was assigned a MC.HCl content of 91.0 % m/m. Using the MC.HCl house standard as a reference, the content of the official standards was compared by LC. Table 2 summarizes the results obtained. The MC.HCl content was determined by comparison with the chromatograms for the MC.HCl house standard, obtained on the same day. A reference solution prepared with MC.HCl house standard at a concentration of 0.01 mg/ml, corresponding to 1.0 % m/m, was used to determine the content of the related substances. The RSD values given in parentheses are within acceptable limits for all the determinations. Several impurities of unknown identity were also present in the standards, but always at a level < 0.1 %. The house standard was less pure than the official standards and the Lederle standard, which were all three of very similar composition. The total mass for the USP-RS was well explained by the results. For the two other standards, the water content was not available. For the BP-CRS the MC.HCl content found was somewhat lower than that declared. For the USP-RS and L-WS the MC content corresponded quite well with the declared potency in μg/mg. However, it should be emphasized that the micrograms mentioned have to be interpreted as micrograms of activity.

Analysis of Commercial Samples

The commercial samples were analysed as described in experimental and the results were calculated as

TABLE 3
Composition of Bulk Samples of Minocycline Hydrochloride

Sample origin and number	7-DDMMC	7-MDMMC	UNK6	EMC	UNK8	UNK9	6-DODMTC	UNK11	9-MC	MC	KF (n;RSD)	Total
A1	0.14 (6)	0.46 (2)	0.07 (10)	0.81 (3)	<0.02	<0.02	0.23 (5)	0.13 (2)	0.06 (37)	91.4 (0.9)	7.2 (4;0.9)	100.5
A2	0.02 (28)	0.16 (10)	0.14 (5)	1.03 (9)	<0.02	0.09 (15)	0.49 (8)	<0.02	0.04 (17)	90.7 (0.8)	8.1 (3;0.9)	100.8
A3	0.01 (17)	0.28 (1)	<0.01	1.24 (4)	0.15 (6)	0.13 (7)	0.33 (7)	0.27 (4)	<0.03	88.9 (1.1)	7.8 (3;0.6)	99.1
B1	0.12 (6)	0.43 (1)	0.10 (13)	0.70 (5)	<0.02	<0.02	0.35 (22)	0.13 (12)	0.04 (27)	91.4 (0.4)	7.5 (3;0.9)	100.8
C	0.18 (19)	0.22 (13)	0.10 (37)	1.57 (4)	<0.02	0.17 (26)	0.37 (25)	<0.02	<0.03	89.2 (0.5)	7.6 (4:1.3)	99.4

Values in % m/m. LC results expressed in terms of MC.HCl. Four independent analyses were performed within one day. RSD (%) values are given in parentheses. UNK = unknown, the number corresponds to the peak number

TABLE 4
Composition of Specialties

Sample	Sample age (year)	7-DDMMC	7-MDMMC	UNK6	EMC	UNK8	UNK9	6-DODMTC	UNK11	9-MC	MC	Total
Capsule	1	0.09	0.41	0.07	0.77	<0.02	<0.02	0.18	0.09	0.07	104.4 (0.8)	106.1
Capsule	6	0.03	0.38	0.10	1.14	0.08	0.13	0.61	0.26	0.07	99.7 (0.9)	102.5
Capsule	12	0.05	0.22	0.07	1.54	0.13	0.14	1.38	0.31	0.08	110.4 (0.7)	114.3
Tablet	1	0.03	0.19	0.06	1.13	0.08	0.11	0.57	0.20	0.07	106.4 (0.1)	108.8

Values in % m/m calculated against the labeled amount and expressed in terms of MC.HCl. Four independent analyses were performed within one day. RSD (%) values are given in parentheses. UNK = unknown, the number corresponds to the peak number in fig. 5. All samples were from manufacturer A.

described above for the standards. Table 3 shows results for bulk samples. The repeatability of the assay is good. EMC, 6-DODMTC and 7-MDMMC are the main impurities. 7-DDMMC, 9-MC and several impurities of unknown identity can also be present. The total mass is well explained. Official monographs limit the MC.HCl content to 96.0-102.5 % m/m, the water content to 4.3-8.0 %, the EMC content to 1.2 % and the sum of other impurities, determined by LC, to 2.0 % [2-4]. The samples examined generally comply with these limits, but some results are slightly over the limit. Sample A2 was found to contain 8.1 % of water. It should be emphasized that minocycline hydrochloride is hygroscopic. The somewhat high value might be due to the handling conditions. The official texts do not mention the hygroscopic character. Sample A3 contains 1.24 % of EMC. In sample C the limit for EMC is clearly exceeded. It is not clear why a limit of 1.2 % was introduced in the official monographs. The BP formerly mentioned 1.4 %, the USP 2.0 %. EMC is not described to be toxic. For the very analogous tetracycline hydrochloride the BP and the Ph. Fr. allow 5 % of 4-epimer while the USP does not prescribe a limit at all.

Table 4 shows the results obtained for specialties, by using the method described for bulk samples. No supplementary validation was carried out, even the selectivity towards excipients was not examined. The repeatability for assay is good. RSD values for the impurities were comparable to those in Table 3 and were not mentioned here. The USP limits the content in capsules to 90.0-115.0 of the labeled amount. Impurities are not specifically limited. The purity profile of these specialties is comparable to that of the bulk samples. Even a twelve years old sample does not show significant degradation. This proves the good stability of MC.HCl in

a solid matrix. The period of validity for these samples was mentioned to be 3 years.

CONCLUSION

The results obtained have shown that the described LC method is highly selective towards minocycline and its related substances. The method is suitable for quantitative analysis of minocycline in bulk samples and can also be used for analysis of preparations. An important advantage of the method is the applicability to the different poly(styrene-divinylbenzene) stationary phases available, which may considerably improve the reproducibility of the method. This is often not obtained with silica-based reversed-phase materials, of which it is known that important differences in selectivity can exist between brands.

ACKNOWLEDGMENTS

The National Fund for Scientific Research (Belgium) is acknowledged for financial support. Lederle-Cyanamid and the Ministry of Health (Belgium) are acknowledged for the gift of samples. Sun Hua is thanked for technical assistance. A. Decoux and I. Quintens are acknowledged for editorial assistance.

REFERENCES

1. Mitscher, L.A., The Chemistry of the Tetracycline Antibiotics, Marcel Dekker, New York, 1978.

2. The United States Pharmacopeia XXII, Supplement IV, United States Pharmacopeial Convention, Rockville, MD, 1991, p. 2479.

3. British Pharmacopoeia 1988, HMSO, London, p. 377.

4. Pharmacopée Française, 10e Edition, Maisonneuve, Sainte-Ruffine, France, 1992.

5. Naidong Weng, Vermeulen, K., Roets, E. and Hoogmartens, J., Evaluation of Analytical Methods. LC of Minocycline, Chromatographia, submitted.

6. Dihuidi, K., Kucharski, M.J., Roets, E., Hoogmartens, J. and Vanderhaeghe, H., Quantitative analysis of doxycycline and related substances by HPLC, J. Chromatogr., 325, 413, 1985.

7. Hoogmartens, J., Melamed, R., Miller, J., van der Vlies, C. and Vanderhaeghe, H., Collaborative study on an improved analysis of doxycycline by liquid chromatography, Pharmeuropa, 1, 39, 1988.

8. Hoogmartens, J., Khan, N.H., Vanderhaeghe, H., van der Leeden, A.L., Oosterbaan, M., Veld-Tulp, G.L., Plugge, W., van der Vlies, C., Mialanne, D., Melamed, R. and Miller, J.H. McB., A collaborative study of the analysis of doxycycline hyclate by high-performance liquid chromatography on polystyrene-divinylbenzene packing materials, J. Pharm. Biomed. Anal., 7, 601, 1989.

9. Wolfs, K., Roets, E., Hoogmartens, J. and Vanderhaeghe, H., Separation of tetracycline and related substances by HPLC on poly(styrene-divinylbenzene), J. Chromatogr., 358, 444, 1986.

10. Khan, N.H., Wera, P., Roets, E. and Hoogmartens, J., Quantitative analysis of tetracycline by high-performance liquid chromatography on polystyrene-divinylbenzene packing materials, J. Liq. Chromatogr., 13, 1351, 1990.

11. Khan, N.H., Roets, E., Hoogmartens, J. and Vanderhaeghe, H., Quantitative analysis of oxytetracycline and related substances by HPLC, J. Chromatogr., 405, 229, 1987.

12. Hoogmartens, J., Naidong Weng, Khan, N.H., Malley, A., Hearty, U., Melamed, R., Gousset, J.P., Creed, P., Woollam, C., Miller, J.H. McB., Fuchs, J. and Vanderhaeghe, H., Collaborative study of the analy-

sis of oxytetracycline and oxytetracycline hydrochloride by liquid chromatography, Pharmeuropa, 2, 77, 1990.

13. Naidong Weng, Roets, E. and Hoogmartens, J., Quantitative analysis of demeclocycline by high-performance liquid chromatography, J. Pharm. Biomed. Anal., 7, 1691, 1989.

14. Naidong Weng, Verresen, K., Roets, E. and Hoogmartens, J., Determination of metacycline and related substances by column liquid chromatography on poly(styrene-divinylbenzene), J. Chromatogr., 586, 61, 1991.

15. Naidong Weng, Cachet, Th., Roets, E. and Hoogmartens, J., Identification of tetracyclines by TLC, J. Planar Chromatogr., 28, 424, 1989.

16. Naidong Weng, Roets, E. and Hoogmartens, J., High performance liquid chromatography of chlortetracycline and related substances on poly(styrene-divinylbenzene)copolymer, Chromatographia, 30, 105, 1990.

17. Dawkins, J.V., Lloyd, L.L. and Warner, F.P., Chromatographic characteristics of polymer-based high-performance liquid chromatography packings, J. Chromatogr., 352, 157, 1986.

18. Paesen, J., Roets, E. and Hoogmartens, J., Liquid chromatography of erythromycin A and related substances on poly(styrene-divinylbenzene), Chromatographia, 32, 162, 1991.

19. Wright, W.W., United States Pharmacopeia, Rockville, MD, Personal communication.