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Quantitative analysis of demeclocycline by high-performance liquid chromatography*

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Abstract: A high-performance liquid chromatographic (HPLC) method suitable for the quality control of demeclocycline is described. The stationary phase is a poly(styrene–divinylbenzene) copolymer, kept at 60°C. The mobile phase comprises 2-methyl-2-propanol–0.2 M potassium phosphate buffer (pH 9.0)–0.02 M tetrabutylammonium hydrogen sulphate (pH 9.0)–0.01 M sodium edetate (pH 9.0)–water (8:10:15:10:57, m/v/v/v/v). The flow rate is 1 ml min^{-1} and detection is performed at 254 nm. Official standards are compared and results for the analysis of a number of commercial bulk samples and preparations are presented. 4-Epidemeclocycline and demethyltetracycline are the main impurities. 4-Epidemethyltetracycline and 2-acetyl-2-decarboxamido-demeclocycline can also be present.

Keywords: *Demeclocycline; high-performance liquid chromatography; poly(styrene–divinylbenzene)copolymer.*

Introduction

In this study a high-performance liquid chromatographic (HPLC) method for the quality control of demeclocycline (DMCTC) is presented. DMCTC is a member of the tetracyclines (TCs), an important group of antibiotics which are widely used. The method presented here is based on previous work concerning the analysis of doxycycline (DOX) [1–3], tetracycline (TC) [4, 5] and oxytetracycline (OTC) [6].

The structure of DMCTC, its degradation products and fermentation impurities are shown in Fig. 1. DMCTC like other tetracyclines undergoes epimerization at position C-4, resulting in the formation of 4-epidemeclocycline (EDMCTC). Demethyltetracycline (DMTC) is described as a fermentation impurity of DMCTC [7]. Because of the secondary hydroxyl group at C-6, which is less likely to be involved in dehydration than is a tertiary hydroxyl group, DMCTC is less liable than other TCs towards acid degradation to form anhydrodemeclocyline (ADMCTC). DMTC is degraded by the same scheme as that shown for DMCTC. Since the 2-acetyl-2-decarboxamido derivatives of TC, OTC and DOX have already been found as fermentation impurities [2–6], it is reasonable to believe that commercial DMCTC can contain 2-acetyl-2-decarboxamidodemeclocycline

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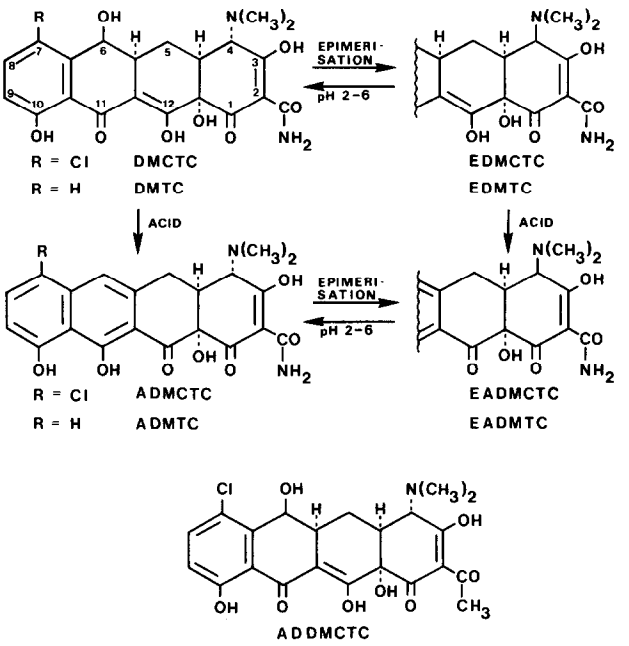

Figure 1
Structure of DMCTC, its degradation products and fermentation impurities.

(ADDMCTC) as well. Therefore a good HPLC method for the quality control of DMCTC should separate 4-epidemethyltetracycline (EDMTC), DMTC, EDMCTC, DMCTC and ADDMCTC; the separation of 4-epianhydrodemethyltetracycline (EADMTC), anhydrodemethyltetracycline (ADMTC), 4-epianhydrodemeclocycline (EADMCTC) and ADMCTC is less important.

Only a few papers have mentioned the separation of DMCTC from some of its potential impurities. DMTC, the most important impurity of DMCTC, has not been mentioned in these reports. In 1982 Hermansson *et al.* [8] reported the separation of DMCTC from EDMCTC, EADMCTC and ADMCTC by using Lichrosorb RP-8 and a mobile phase at pH 8.0; no chromatogram was shown. Mobile phases at slightly alkaline pH are known to reduce the lifetime of the silica based reversed-phase stationary phase [9]. In 1986, Reeuwijk and Tjaden [10] reported the separation of DMCTC from EDMCTC, EADMCTC and ADMCTC. According to the chromatogram shown, the amount of DMCTC in the mixture compared with the amount of impurities is very low. EDMCTC will not be separated adequately from DMCTC if the quantitative analysis of DMCTC is performed whereby a small amount of EDMCTC has to be separated from a big DMCTC peak. It can be concluded that up to now no good HPLC method for the quality control of DMCTC has been published.

Experimental

Reagents and samples

Organic solvents were from Janssen Chimica (Beerse, Belgium). Tetrahydrofuran (THF) was distilled after the control of absence of peroxides. Tetrabutylammonium

(TBA) hydrogen sulphate was from the same manufacturer. Other reagents were of *pro analysi* quality (Merck, Darmstadt, FRG). Water was distilled twice from glass apparatus.

The United States Pharmacopeia Reference Standard Lot G (1000 μg mg^{-1}) (USP-RS), the European Pharmacopoeia Chemical Reference Substance Lot 1 (988 IU mg^{-1}; Ph. Eur.-CRS), and the WHO International Standard (1000 IU mg^{-1} WHO -IS) were available. All these reference substances are hydrochloride salts (DMCTC·HCl). 2-Acetyl-2-decarboxamidotetracycline hydrochloride (ADTC·HCl) was kindly donated by Pfizer (Groton, CT, USA).

Bulk samples of DMCTC·HCl were obtained from one manufacturer. Capsules and ointments of the drug produced by the same manufacturer were obtained from the Belgian market.

House standards of DMCTC·HCl (98.8%), EDMCTC·HCl (96.5%) and DMTC dihydrate (97.2%), this content (m/m) being expressed in terms of the hydrochloride salt, were prepared. The preparation of these house standards will be described elsewhere. Small amounts of EDMTC·HCl, ADMCTC·HCl, EADMCTC·HCl, ADMTC·HCl and EADMTC·HCl were also prepared but the purity of these compounds was not precisely determined since they are only minor impurities.

Columns

The poly(styrene–divinylbenzene)copolymers (PSDVB) PLRP-S (8 μm, 100 Å; Polymer Labs, Church Stretton, Shropshire, UK), PRP-1(10 μm; Hamilton, Reno, NV, USA), RoGel (7–9 μm; RSL-Alltech Europe, Eke, Belgium) and TSK-gel (10 μm; Toyo Soda, Tokyo, Japan) were packed in 250 × 4.6 mm i.d. columns by a published method [4].

HPLC apparatus and operating conditions

The HPLC apparatus consisted of a SP 8700 XR, three solvent delivery system (Spectra Physics, San Jose, CA, USA), an injector model CV-6-UHPa-N60 (Valco, Houston, TX, USA) equipped with a 20 μl loop, a 254 nm fixed wavelength detector Model 440 (Waters Associates, Milford, MA, USA) and an integrator Model 3390 A (Hewlett–Packard, Avondale, PA, USA). For the identification of ADDMCTC, a Waters Model 990 photodiode array detector was used. The column was immersed in a waterbath at 60°C and the flow rate was 1.0 ml min^{-1}. Each evening the pump was washed with methanol–water (50:50, v/v). The back pressure was 900–1400 psi depending on the brand of packing material.

Mobile phase

The mobile phase finally used for analysis was prepared as follows: the required amount of 2-methyl-2-propanol was weighed and rinsed into a volumetric flask with 200 ml of water; depending upon the brand of packing material employed, 5.7 to 8.0%, m/v, of 2-methyl-2-propanol was used. To this mixture was added 100 ml of 0.2 M potassium phosphate buffer (pH 9.0), 150 ml of 0.02 M TBA hydrogen sulphate and 100 ml of 0.01 M sodium edetate (EDTA). During preparation of the latter two solutions, the pH was adjusted to 9.0 with sodium hydroxide solution. The volume was made up to 1000 ml with water. Mobile phases were degassed by sonication. The 0.2 M potassium phosphate buffer (pH 9.0) was prepared by mixing 0.2 M potassium monohydrogen phosphate and 0.2 M potassium dihydrogen phosphate. For the purpose

of isocratic analysis of slowly eluted anhydroderivatives, a second mobile phase with an increased amount of organic modifier (11%, m/v, for the RoGel column) was also used.

Sample preparation and stability

About 25.0 mg of bulk sample was precisely weighed, dissolved in 0.01 M hydrochloric acid and diluted to 25.0 ml with the same solvent. For capsules, the sample was weighed to contain the equivalent of about 25.0 mg of DMCTC·HCl and diluted to 25.0 ml with 0.01 M hydrochloric acid. 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 1.5-μm membrane filter. For ointments, a sample equivalent to 25.0 mg DMCTC·HCl was shaken with a mixture of 25 ml of hexane and 25.0 ml of 0.01 M hydrochloric acid. After separation of the layers an aliquot of the aqueous layer was filtered as above.

At room temperature the EDMCTC content in a solution of DMCTC in 0.01 M hydrochloric acid increased from 0.1 to 0.6% in about 3 h. When the solution was stored at 6°C in the dark the EDMCTC content increased from 0.1 to 0.2% in about 3 h.

Results and Discussion

Development of the chromatographic method

Experience obtained with the HPLC analysis of DOX, TC and OTC on PSDVB stationary phases was used for the development of a method for DMCTC [1–6]. As mentioned previously ADMTC, EADMCTC and ADMCTC are less important impurities. In preliminary experiments it was observed that these impurities were well separated from DMCTC and the other impurities, and could not be quantitated in the same isocratic method due to strong retention. Therefore, they were not considered from the beginning of the development of an isocratic HPLC method. Eventual analysis of these impurities should be carried out by a gradient system or by a second isocratic method using a stronger mobile phase.

Among the organic modifiers examined, only THF and 2-methyl-2-propanol gave good separations. The use of THF has some practical disadvantages [4]. Therefore 2-methyl-2-propanol was chosen as the organic modifier. In preliminary experiments, where a mobile phase at pH 9.0 was used, a small peak was eluted right after the DMCTC peak of the commercial sample. In analogy with results previously obtained for TC, OTC and DOX, this impurity was thought to be ADDMCTC [2, 5, 6]. This fermentation impurity has not been described before. Its structure is shown in Fig. 1. The structure proposed for this impurity was confirmed by its UV spectrum obtained by photodiode array detection. This corresponded very well with the UV spectrum of 2-acetyl-2-decarboxamidotetracycline (ADTC) which was available as a reference material and with the UV spectra of the corresponding impurities in samples of OTC, DOX and TC and which had been identified previously as the 2-acetyl derivatives [2, 5, 6]. For 2-acetyl derivatives the ratio of the absorbance at the maximum at about 270 nm to that at about 380 nm is larger than that for the corresponding TC. The 2-acetyl derivatives show a pronounced minimum at about 240 nm, which is absent for the TCs. Both 2-acetyl derivatives and TCs show a minimum at about 320 nm.

Figure 2 shows the influence of the pH on the separation. It is obvious that pH 9.0 is suitable for analysis of DMCTC. At this pH the best separation of DMCTC and ADDMCTC, DMTC and EDMCTC is obtained. Figure 3 shows the influence of the

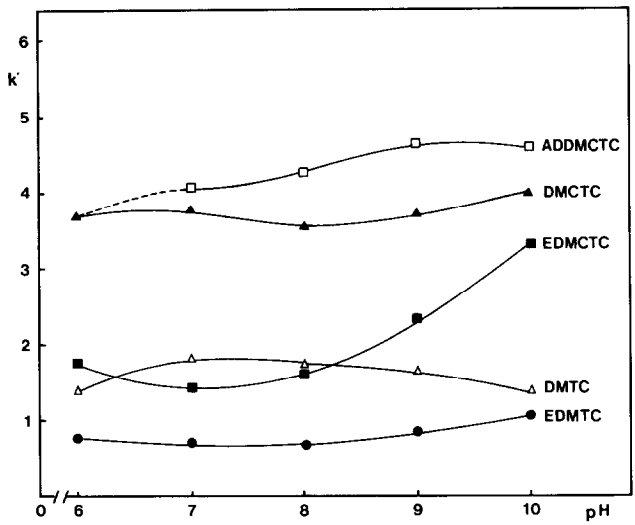

Figure 2
Influence of the pH of the mobile phase on the separation of DMCTC and related substances. Column: RoGel. Mobile phase: 2-methyl-2-propanol (8.0 g)–0.02 M phosphate buffer of the pH indicated (10.0 ml)–0.02 M TBA hydrogen sulphate (15.0 ml)–0.01 M EDTA (10.0 ml). The pH of the latter two solutions was brought to the pH indicated with sodium hydroxide solution. Water was added up to 100.0 ml. See text for other conditions.

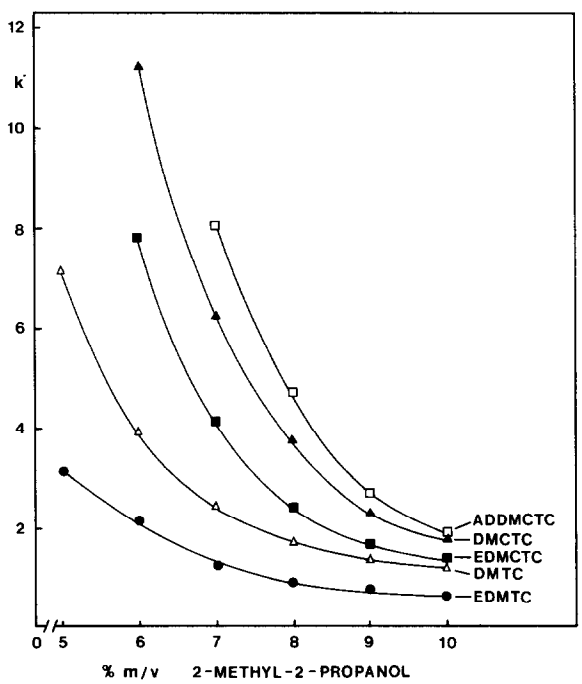

Figure 3
Influence of the concentration of the organic modifier on the separation of DMCTC and related substances. Column: RoGel. Mobile phase: 2-methyl-2-propanol (×g)–0.2 M phosphate buffer (pH 9.0; 10.0 ml)–0.02 M TBA hydrogen sulphate (pH 9.0; 15.0 ml)–0.01 M EDTA (pH 9.0; 10.0 ml)–water (up to 100.0 ml). See text for other conditions.

concentration of 2-methyl-2-propanol on the separation. A mobile phase with 8%, m/v, of 2-methyl-2-propanol was chosen for further study. Figure 4 shows the influence of the concentration of TBA. Finally, 15%, v/v, of 0.02 M TBA was chosen. The influence of the phosphate buffer concentration is minor. Retention decreases with increasing concentration up to a concentration of 10%, v/v, of 0.2 M phosphate buffer. 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 chosen. The presence of EDTA in the mobile phase is necessary, otherwise the separation of DMCTC and ADDMCTC rapidly deteriorates. A proportion of 10%, v/v, of 0.01 M EDTA was used finally.

A column temperature of 60°C was maintained throughout the study. This temperature was also found suitable for analysis of other TCs [1–6]. The stability of DMCTC during analysis was checked by repeated analysis of DMCTC·HCl house standard at 50, 60 or 70°C. The area of the DMCTC peak remained unchanged. The content of EDMCTC, the most easily formed degradation product of DMCTC, was not found to increase with temperature.

Figure 5 shows the chromatograms obtained on several PSDVB copolymers available on the market. A good separation can be obtained on all the columns. Physical properties of the packing materials and column characteristics are indicated in Table 1. All further analyses were performed on a RoGel column. Under the conditions established above ADMTC, EADMCTC and ADMCTC had retention times of about 30, 80 and 160 min, respectively. In order to quantitate these impurities a fast eluting mobile phase containing 11%, m/v, of 2-methyl-2-propanol was used.

Calibration curves and reproducibility

Calibration curves were obtained with the house standards DMCTC·HCl, EDMCTC·HCl and DMTC dihydrate. The following relationships were found, where y = peak area, x = amount of hydrochloride salt injected in micrograms, n = number of

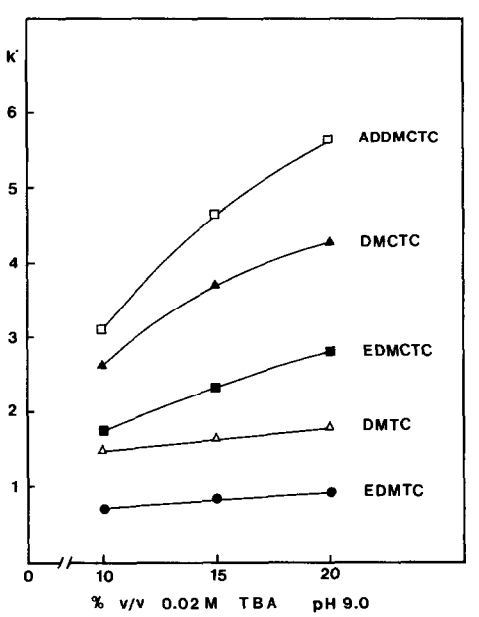

Figure 4
Influence of the concentration of TBA hydrogen sulphate on the separation of DMCTC and related substances. Column: RoGel. Mobile phase: 2-methyl-2-propanol (8.0 g)–0.2 M phosphate buffer (pH 9.0; 10.0 ml)–0.02 M TBA hydrogen sulphate pH 9.0 (×ml/100 ml)–0.01 M EDTA (pH 9.0; 10.0 ml)–water (up to 100.0 ml). See text for other conditions.

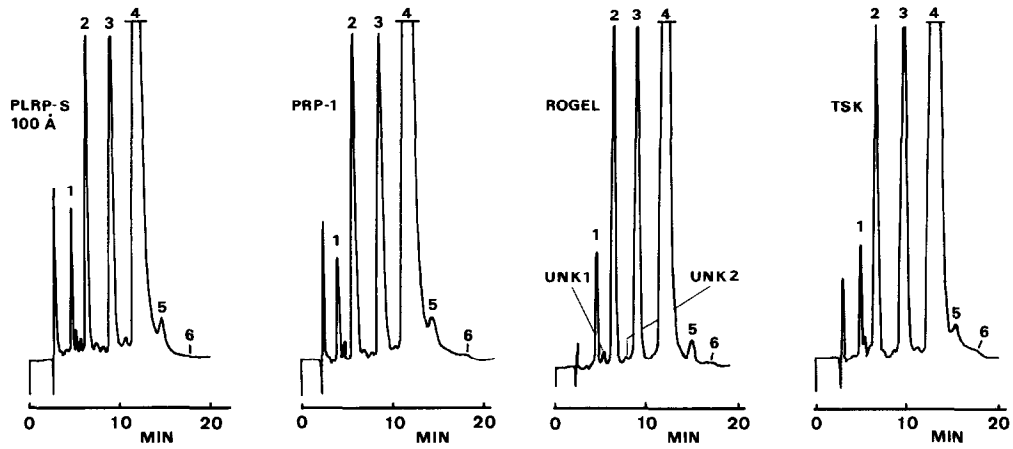

Figure 5
HPLC of DMCTC·HCl on several poly(styrene–divinylbenzene)copolymers. Mobile phase: 2-methyl-2-propanol (×g/100 ml)–0.2 M potassium phosphate buffer (pH 9.0; 10.0 ml)–0.02 M TBA hydrogen sulphate pH 9.0 (15.0 ml)–0.01 M EDTA (pH 9.0; 10.0 ml)–water (up to 100.0 ml). PLRP-S 100 Å (8 μm) x = 6.8, PRP-1 (10 μm) x = 6.5, RoGel (7–9 μm) x = 8.0, TSK-gel (10 μm) x = 5.7. Flow rate: 1 ml min^{-1}. Detection: UV at 254 nm. Temperature: 60°C. 1 = EDMTC (0.9%, m/m), 2 = DMTC (4.6%, m/m), 3 = EDMCTC (5.7%, m/m), 4 = DMCTC, 5 = ADDMCTC (0.4%, m/m), 6 = EADMTC (<0.05%, m/m), UNK = Unknown.

samples analysed, r = correlation coefficient, $S_{y,x}$ = standard error of estimate, CR = range of injected mass examined. DMCTC: $y = 230 + 12168x$, $r = 0.9999$ ($n = 12$), $S_{y,x} = 25$, CR = 16–24 μg; EDMCTC: $y = -20 + 12790x$, $r = 0.9993$ ($n = 12$), $S_{y,x}$ = 120, CR = up to 2 μg; DMTC: $y = 156 + 12564x$, $r = 0.9999$ ($n = 12$), $S_{y,x} = 13$, CR = up to 1.6 μg.

The quantitation limits were 0.01% for EDMTC, DMTC and EDMCTC, and 0.05% for ADDMCTC and EADMTC. EDMTC was expressed in terms of DMTC. Since a reference substance of ADDMCTC was not available the detection limit for ADDMCTC was determined by injecting mixtures of two solutions, one containing a DMCTC·HCl sample with 0.4% ADDMCTC·HCl, the other containing the DMCTC·HCl house standard. The more strongly retained anhydroderivatives ADMTC, EADMCTC and ADMCTC were analysed by a second isocratic method using a mobile phase with more organic modifier. The quantitation limit was 0.05%. EADMTC and ADMTC were expressed in terms of EADMCTC and ADMCTC, respectively. The house standard was analysed 38 times over a period of 4 days. The relative standard deviation (RSD) for DMCTC was 0.5%.

Comparison of DMCTC·HCl standards

The DMCTC·HCl house standard was titrated with perchloric acid in non-aqueous conditions. A total of eight titrations gave a mean of 99.6% DMCTC·HCl (RSD = 0.5%). A total of six Karl Fischer titrations gave a mean of 0.4% water (RSD = 14%). This result was confirmed by loss on drying (see Table 2). The total content of the DMCTC·HCl house standard was therefore accepted to be 99.6% and this figure was corrected by means of chromatography with the aid of the calibration curves for the potential impurities. The total amount of chromatographic impurities corresponded to 0.8%. Therefore, the DMCTC·HCl house standard was assigned a purity of 98.8%.

Table 1
Column characteristics under the conditions mentioned in Fig. 5

Column	Proportion (% m/v) of 2-methyl-2-propanol in mobile phase	Plate number per column (DMCTC)	Peak symmetry (DMCTC)	Resolution (R_s)			Capacity factor (k')			
				EDMTC–DMTC	DMTC–EDMCTC	EDMCTC–DMCTC	EDMTC	DMTC	EDMCTC	DMCTC
PLRP-S 8 μm	6.8	3604	1.1	4.5	4.6	4.4	0.66	1.30	2.18	3.28
PRP-1 10 μm	6.5	1654	1.2	5.0	4.0	3.3	0.74	1.58	2.63	4.01
RoGel 7–9 μm	8.0	2926	1.0	4.5	5.4	3.3	1.00	1.80	3.40	4.31
TSK-gel 10 μm	5.7	2283	1.1	3.8	4.5	3.7	0.66	1.29	2.30	3.53

Table 2
Composition of DMCTC·HCl standards

	House standard	Ph. Eur.-CRS (988 IU mg^{-1})	USP-RS (1000 μg mg^{-1})	WHO-IS (1000 IU mg^{-1})
Number of solutions	38	10	6	4
Number of analyses	38	20	12	8
Number of days	4	2	3	2
EDMTC*	<0.01	<0.01	<0.01	<0.01
UNK 1*	0.03 (23)	0.04 (25)	0.03 (22)	0.03 (13)
DMTC	0.7 (1.7)	0.5 (1.3)	0.4 (2.5)	1.4 (1.6)
EDMCTC	0.1 (16)	1.2 (1.8)	0.5 (6.7)	0.6 (6.0)
DMCTC	98.8 (0.5)	95.4 (0.4)	97.7 (0.4)	97.9 (0.6)
ADDMCTC†	<0.05	0.08 (21)	<0.05	<0.05
EADMTC‡	<0.05	<0.05	<0.05	<0.05
ADMTC§**	<0.05	<0.05	<0.05	<0.05
EADMCTC**	<0.05	<0.05	<0.05	<0.05
ADMCTC**	<0.05	<0.05	<0.05	<0.05
Subtotal	99.6	97.2	98.6	99.9
Non-aqueous titration	99.6	ND	ND	ND
n (RSD)	8 (0.5)			
Water determined (KF)	0.4	2.5	ND	ND
n (RSD)	6 (14)	4 (9.9)		
Loss on drying determined‖	0.3	ND	ND	ND
n (RSD)	2 (1.1)			
Loss on drying declared		0.7 [12]	1.3 [13]	0.0 [14]
Water declared		1.8 [11]		

Values in percent (m/m) expressed in terms of the hydrochloride; RSD values are given in parentheses; *n* = number of analyses; ND = not determined owing to the limited amount of sample; UNK = unknown; KF = Karl Fisher titration.

*Expressed in terms of DMTC·HCl.

†Expresssed in terms of DMCTC·HCl.

‡Expressed in terms of EADMCTC.

§Expressed in terms of ADMCTC.

‖For 3 h *in vacuo* over diphosphorus pentoxide at a pressure <0.1 kPa and a temperature of 60°C.

**Determined by a fast eluting system.

Using the DMCTC·HCl house standard, the content of the official standards was determined by HPLC. Table 2 summarizes the results obtained. The DMCTC·HCl content was determined on several days by comparison with the chromatograms obtained for the DMCTC·HCl house standard on the same days. The impurities were determined using the calibration curves for EDMCTC·HCl and DMTC. Since a reference substance of ADDMCTC was not available ADDMCTC was expressed in terms of DMCTC·HCl using the surface ratio ADDMCTC/DMCTC in each chromatogram. The RSD values, given in parentheses below the content, are within acceptable limits for all the determinations. An unknown impurity (UNK 1) which is eluted right after EDMTC (see Fig. 5) is present in all the standards. EDMTC, EADMTC, ADMTC, EADMCTC and ADMCTC are below the detection limits for all the standards. Non-aqueous titration and loss on drying measurements were not carried out for the official standards, owing to the limited amount of sample available.

For the Ph.Eur.-CRS, the subtotal of DMCTC·HCl and impurities is lower than that of other standards. The Ph.Eur.-CRS therefore is less pure than the WHO-IS and the difference in content seems to be more important than is reflected by the difference in international units. The declared content for the USP-RS of 1000 μg mg^{-1} is an overestimate, since it contains only 97.7% of DMCTC·HCl and 0.4% of DMTC·HCl. In

comparison, the house standard would contain more than 1000 μg mg^{-1}, which is theoretically impossible if the micrograms are interpreted as mass units. In fact, the micrograms reported by the USP have to be interpreted as micrograms of activity. This can be a source of confusion [15, 16]. For all the standards the sum of the subtotal and the water content or loss on drying is very close to the theoretical value. For the Ph.Eur.-CRS the available information is contradictory. The water content reported by the manufacturer of the standard is 1.8% [11], whereas the loss on drying reported by the Ph.Eur. Laboratory is 0.7% [12]. In the authors' laboratory the water content of this standard was found to be 2.5%. This value matches best with the subtotal of 97.2%. The loss on drying for the WHO-IS is reported to be zero since a completely dry substance has been packed in the ampoules [14].

Analysis of commercial samples

The commercial samples were analysed as described above for the standards. All the samples contained the hydrochloride salt of DMCTC. Table 3 shows results for the bulk samples. The reproducibility of the DMCTC assay is very good. Only very small amounts of ADMTC, EADMCTC and ADMCTC were found in a sample more than 25 years old. This means that DMCTC·HCl is quite stable towards acid degradation. The stability

Table 3
Composition of bulk samples of DMCTC·HCl

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
Age of the sample (months)	NM	NM	9	300
Content declared	93.0%	91.2%	91.2%	NM
Number of solutions	6	7	7	7
Number of analyses	6	7	7	7
Number of days	2	3	3	3
EDMTC*	0.07 (2.2)	0.2 (5.8)	0.09 (9.4)	0.9 (2.7)
UNK 1*	0.1 (0.4)	0.1 (2.1)	0.1 (4.0)	0.2 (4.3)
DMTC	2.7 (3.7)	3.0 (3.4)	2.4 (3.3)	4.6 (3.2)
UNK 2†	0.04 (28)	0.2 (15)	0.1 (8.5)	0.1 (17)
EDMCTC	3.0 (0.7)	4.3 (1.5)	3.6 (0.9)	5.7 (1.0)
DMCTC	90.7 (0.6)	88.2 (0.5)	89.6 (0.6)	83.3 (0.3)
ADDMCTC‡	<0.05	<0.05	<0.05	0.4 (18)
EADMTC§	<0.05	<0.05	<0.05	<0.05
ADMTC‖††	<0.05	<0.05	<0.05	0.05
EADMCTC††	<0.05	<0.05	<0.05	0.05
ADMCTC††	0.05	0.07	0.05	0.07
Subtotal %	96.7	96.1	95.9	95.4
Water determined (KF)	2.8	3.3	2.8	2.8
n (RSD)	3 (6.0)	3 (2.3)	3 (2.3)	3 (1.4)
Loss on drying**	2.2	2.8	2.5	ND
n (RSD)	2 (0.1)	2 (0.5)	2 (1.2)	
Total	99.5	99.4	98.7	98.2

Values in percent (m/m) expressed in terms of the hydrochloride; RSD values are given in parentheses; NM = not mentioned; UNK = unknown; *n* = number of determinations; KF = Karl Fisher titration; ND = not determined due to limited amount of sample available.

*Expressed in terms of DMTC·HCl.

†Expresssed in terms of EDMCTC·HCl.

‡Expressed in terms of DMCTC.

§Expressed in terms of EADMCTC.

‖Expressed in terms of ADMCTC.

**For 3 h *in vacuo* over diphosphorus pentoxide at a pressure <0.1 kPa and a temperature of 60°C.

††Determined by a fast eluting system.

Table 4
Composition of preparations of DMCTC

Sample number	Preparation	Sample age (months)	Number of independent analyses	EDMTC*	UNK 1*	DMTC	UNK 2†	EDMCTC	ADDMCTC‡	DMCTC Mean	DMCTC RSD
5	Capsules	33	3	0.2	0.1	3.4	0.08	4.5	<0.05	94.9	0.2
6	Capsules	35	3	0.2	0.1	3.5	0.08	4.4	<0.05	93.9	0.3
7	Capsules	33	3	0.2	0.1	3.5	0.08	4.4	<0.05	93.8	0.6
8	Capsules	35	3	0.2	0.1	3.5	0.08	4.5	<0.05	95.3	0.2
9	Capsules	3	3	0.1	0.1	2.6	0.05	4.1	<0.05	102.4	0.2
10	Ointment	61	3	0.3	0.2	2.7	0.02	7.5	<0.05	91.6	0.7
11	Ointment	74	2	3.2	<0.01	1.0	<0.01	90.7	<0.05	5.0	3.9

Values in percent (m/m) of label claim of DMCTC expressed in terms of the hydrochloride.
*Expressed in terms of DMTC·HCl.
†Expressed in terms of EDMCTC·HCl.
‡Expressed in terms of DMCTC·HCl.

of DMCTC is probably not only due to the secondary hydroxyl group at C-6 [7, 17] but also the chlorine group at C-7 since it was found that DMTC·HCl can form ADMTC·HCl more easily. The same is true for the pair CTC·HCl and TC·HCl where CTC·HCl is much more stable towards acid degradation than TC·HCl [18]. Besides the UNK 1, already mentioned, another impurity of unknown identity (UNK 2), which is eluted before EMDCTC, is present in all the samples. UNK 2 is expressed in terms of EDMCTC.

The water content for all the bulk samples was beyond the Ph.Eur. [19] and USP [20] limit of 2%. The results obtained by loss on drying, which is the method prescribed by the official texts, were quite well confirmed by Karl Fischer titration. Results obtained by loss on drying are always somewhat lower than those obtained by Karl Fischer titration. A prolongation of the drying period up to 7 h did not increase the results obtained for loss on drying.

Table 4 gives the composition of some capsules and ointments. DMCTC·HCl in capsules is quite stable. The period of storage has no significant influence on the stability of DMCTC·HCl. For all the capsules the content of EADMTC, ADMTC or EADMCTC is <0.05%, and the content of ADMCTC is <0.1%. DMCTC·HCl in ointments is less stable. More EDMCTC was found in ointments than in bulk samples or capsules. Owing to the interference of other ingredients in ointments small amounts of anhydroderivatives could not be precisely measured. It was surprising to find more than 90% of EDMCTC·HCl in an ointment preparation, whereas another ointment preparation of the same brand and of nearly the same age contained only 7.5% of EDMCTC·HCl. The storage conditions of these two ointments were similar. Therefore, the difference between these two preparations might be an indication that the manufacturing process plays an important rôle in the stability of the incorporated DMCTC·HCl. It should be mentioned that at the moment of analysis both these ointments had exceeded the specified limit of shelf-life.

Conclusions

The results have shown that the HPLC method is very suitable for the quantitative analysis of DMCTC in bulk samples and in preparations. Since DMCTC is quite stable towards acid degradation it seems unnecessary in routine analysis to provide a second isocratic system to determine the anhydroderivatives. An important advantage of the method is the applicability to the different polymer materials available on the market. This is usually not obtained with silica-based reversed-phase materials for which it is known that important differences in selectivity can exist between brands.

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