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Quantitative analysis of chlortetracycline and related substances by high-performance liquid chromatography*

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Abstract Isocratic high-performance liquid chromatography on Zorbax C8 7 μm allows quantitative determination of chlortetracycline, 4-epichlortetracycline, tetracycline, demethylchlortetracycline and isochlortetracycline using a mobile phase containing dimethylsulphoxide, 1 M perchloric acid and water (35 5 60) The minor impurities anhydrochlortetracycline and 4-epianhydrochlortetracycline, which are more strongly retained can be determined using a second isocratic system with a mobile phase containing more organic modifier The method has been used for the comparison of official standards and for the analysis of a number of commercial samples

Keywords *Chlortetracycline, reversed-phase chromatography*

Introduction

Chlortetracycline (CTC) is the oldest antibiotic of the tetracycline (TC) group CTC like other tetracyclines, undergoes epimerization at position C-4 forming 4-epichlortetracycline (ECTC) [1] Due to the hydroxyl group at C-6, CTC is liable to acid degradation forming anhydrochlortetracycline (ACTC) [2, 3] ACTC further epimerizes at position C-4 resulting in the formation of 4-epianhydrochlortetracycline (EACTC) CTC unlike other tetracyclines, is not stable in alkaline medium [3, 4] Alkaline decomposition results in the formation of isochlortetracycline (ISOCTC) which further epimerizes to 4-epiisochlortetracycline (EISOCTC) [1], see Fig 1 Some TC and 6-demethylchlortetracycline (DMCTC) may also be present in CTC samples [5] Therefore a good method for purity control should separate TC, DMCTC, ISOCTC, EACTC, ACTC, ECTC and CTC The separation of the 4-epimers ETC, EISOCTC, EDMCTC and of anhydrotetracycline (ATC), epianhydrotetracycline (EATC) and 6-demethyltetracycline (DMTC) is of minor importance since they can be considered as impurities of impurities

Hermansson *et al* reported separations of CTC and its related impurities on reversed-phase material, no chromatogram was shown, but k' values were mentioned for TC

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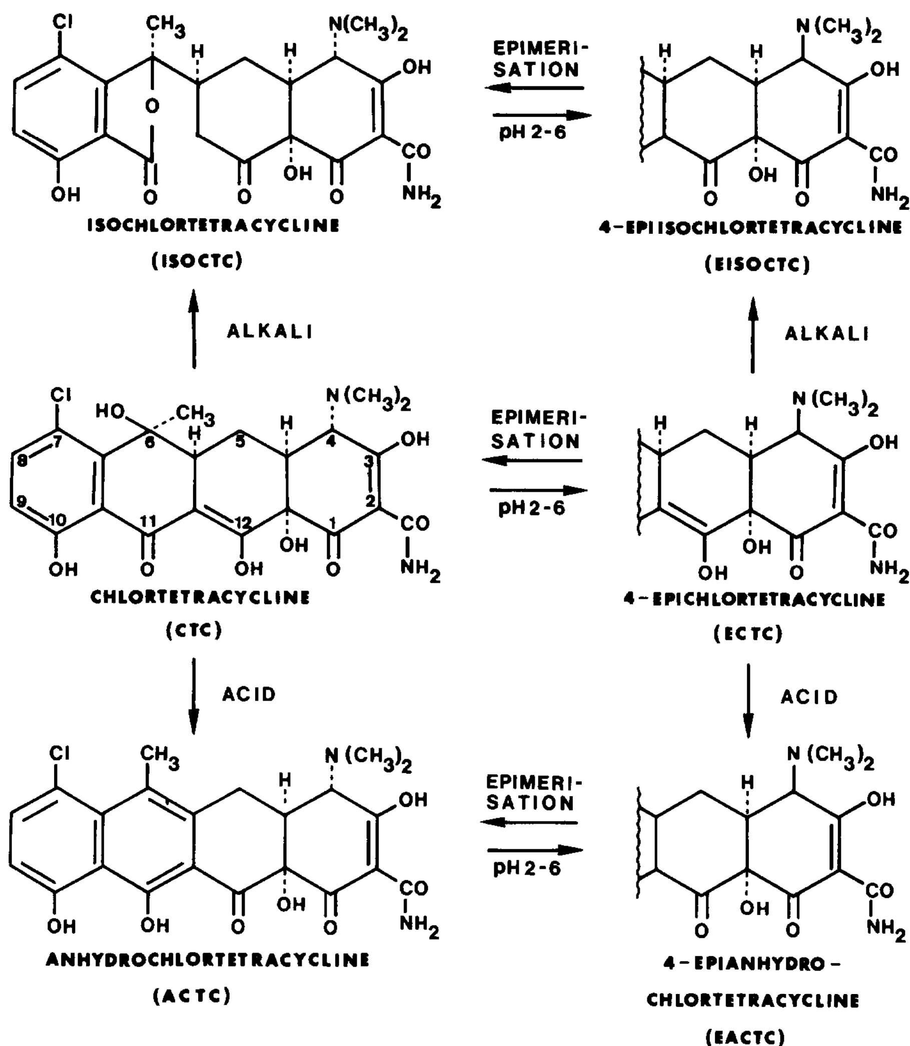

Figure 1
Structures of chlortetracycline and its degradation products

(1 3), ECTC (1 5), DMCTC (1 9), CTC (2 6), ISOCTC (3 9), EACTC (11 1) and ACTC (48 9) [6] The major disadvantage of this method is the pH (8 0) of the mobile phase, which is harmful for reversed-phase materials Whether CTC would degrade to ISOCTC during HPLC at pH 8 0 at room temperature, is not known It is not sure whether, with the k' values mentioned, a small DMCTC peak would separate from a broad CTC peak Another disadvantage is the very long retention of ACTC The same year, Aszalos *et al* published a method using a mobile phase at pH 6 6 which allowed the separation of ECTC, TC and CTC with retention times (min) of 2, 2 5 and 7 5, respectively [7] The peak identified as ECTC probably did not correspond to the 4-epimer of CTC As is observed from other chromatograms in the same paper, 4-epimers are not separated as well as indicated and the small peak before TC probably corresponded to ETC instead of ECTC There was no clear baseline separation between

TC and CTC which was probably due to the presence of ECTC In many of the chromatograms from the literature, showing the separation of mixtures of TCs, the CTC peak is preceded by a small, not well shaped and only partly resolved, peak which is believed by us to correspond to ECTC

Later on Howell *et al* described a method, said to separate ISOCTC, ECTC, CTC, EACTC and ACTC [8] There was incomplete separation between ISOCTC, ECTC and CTC The anhydroderivatives eluted much later The separation of TC, being the main impurity of CTC, was not mentioned It is clear that this method is not suitable for purity control of CTC

More recently a paper was published describing the separation of CTC and its related compounds using poly(styrene–divinylbenzene) copolymer (PSDVB) material as the stationary phase [9] The separation of small amounts of ISOCTC, ECTC and CTC was incomplete If, for the purpose of purity, control CTC is injected in larger amounts, separation of ISOCTC and ECTC from the main peak cannot be obtained Moreover, the separation of TC was not mentioned and the anhydroderivatives were so strongly retained that detection of small amounts was not possible

The isocratic method using reversed-phase material described in this paper, enabled the separation of CTC, ECTC, TC, DMCTC and ISOCTC, ACTC and EACTC The latter two were strongly retained so that for quantitative determination of these impurities, a mobile phase containing more organic modifier had to be used

Experimental

Samples and reference substances

The following standards were compared the United States Pharmacopeia Reference Standards, Lot H-1 (1000 $\mu g\ mg^{-1}$, USP-RS H-1) and Lot I-1 (1007 $\mu g\ mg^{-1}$, USP-RS I-1), the European Pharmacopoeia Chemical Reference Substance (1000 i u mg^{-1}, Ph Eur -CRS Lot 1), the WHO Second International Standard (1000 i u mg^{-1}, WHO-IS) and an Italian Reference Standard CT-001 (1007 i u mg^{-1}, Ital -RS) from the Istituto Superiore di Sanita, Rome

Some of the bulk samples examined were of known origin (Belgium, Spain, Peoples' Republic of China and USA), while others were of unknown origin, being obtained from wholesalers The specialities were obtained from the Belgian market The manufacturer of the incorporated CTC was often not known

Reference substances of CTC HCl (house standard), ECTC HCl, TC HCl, ISOCTC HCl, ACTC HCl, EACTC HCl and ATC HCl were provided from Janssen Chimica (Beerse, Belgium) A sample of DMCTC HCl was kindly donated by Cyanamid GmbH (Wolfratshausen, FRG) DMTC was prepared from DMCTC by hydrogenation

Solvents and reagents

Organic solvents were from Janssen Chimica and Merck (Darmstadt, FRG) Tetrahydrofuran was distilled after control for absence of peroxides Perchloric acid (70% m/v) purissimum was from UCB (Brussels, Belgium) Water was freshly distilled from glass apparatus

HPLC apparatus and operating conditions

Isocratic elution was used throughout the study 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 280 nm fixed wavelength detector Model 440 (Waters Associates, Milford, MA, USA) and an integrator Model 3393 A (Hewlett–Packard, Avondale, PA, USA) The columns (250 × 4 6 mm) were packed in the laboratory with reversed-phase packing materials of different origin Zorbax from Du Pont (Wilmington, DE, USA), Nucleosil from Macherey-Nagel (Duren, FRG), Partisil from Whatman (Clifton, NJ, USA), Spherisorb from Phase Separations (Queensferry, Clwyd, UK), RSil and RoSil from Alltech Europe (Eke, Belgium), LiChrosorb from Merck, μ-Bondapak from Waters A PSDVB material, Rogel, was obtained from Alltech Table 1 shows further information on these columns The columns were packed in the laboratory by previously described methods [10, 11] The columns were immersed in a waterbath at 35°C and the flow rate was 1 0 ml min^{-1} Each evening the column was washed with about 20 ml of methanol The back pressure was between 1000–2500 psi depending upon the amount of organic modifier in the mobile phase

For the final analysis of official standards and samples, two mobile phases with different amounts of dimethylsulphoxide (DMSO) as the organic modifier were used These mobile phases further contained 5% v/v of 1 M perchloric acid and water For the analysis of CTC and fast eluted related substances, 32% v/v of DMSO was used (Mobile phase A) while for the analysis of slow eluted anhydroderivatives, 60% v/v of DMS0 was used (Mobile phase B) The mobile phases were degassed by sonication and flushed with helium before use

Sample preparation and stability

For all samples, weights equivalent to 25 mg of CTC HCl were taken Bulk samples were dissolved and diluted to 25 0 ml with 0 01 M HCl For capsules and tablets,

Table 1
Column characteristics

Column number	Packing material	Column age (weeks)	Particle size (μm)	Particle shape	x
I	Zorbax C8	201	7	S	14
II	Zorbax C8	28	7	S	50
III	Zorbax C8	5	7	S	50
IV	Zorbax C8	1	7	S	50
V	Nucleosil C8	104	10	S	32
VI	Partisil CCS/C8	29	10	I	20
VII	Spherisorb C8	54	5	S	41
VIII	Spherisorb C8	26	5	S	45
IX	RoSil C8	1	8	S	50
X	Partisil ODS	31	10	I	27
XI	Partisil ODS	2	10	I	24
XII	Partisil ODS 3	1	10	I	50
XIII	Zorbax C18	123	7	S	41
XIV	Spherisorb ODS 1	6	10	S	46
XV	Spherisorb ODS 2	5	10	S	51
XVI	μ-Bondapak C18	64	10	I	29
XVII	LiChrosorb RP18	1	10	I	53
XVIII	RoSil C18 LL	25	10	I	44
XIX	RSil CN	22	10	S	5
XX	Rogel	20	7–9	S	55

I = irregular, S = spherical, column length = 250 × 4 6 mm, i d , x = percentage v/v of DMSO in the mobile phase used to obtain the results shown in Fig 2

samples were diluted to 25 0 ml with 0 01 M HCl, sonicated for 5 min at room temperature and then centrifuged at 2500*g* for 5 min The supernatant was filtered through a membrane filter with 1 2 μm pores For ointments, the sample was shaken with a mixture of 20 ml of hexane and 20 0 ml of 0 01 M HCl After separation of the layers, an aliquot of the aqueous layer was filtered as mentioned above For pressurized sprays, the spray can was cooled in a mixture of *n*-butanol and solid carbon dioxide, decapped and stored overnight to evaporate slowly the volatile components The residue was taken up in 0 01 M HCl to obtain the required concentration If necessary, the mixture was shaken with chloroform to remove lipophilic spray components

Over a period of 5 h at room temperature, the ECTC content of a solution of CTC in 0 01 M HCl, stored in daylight or in the dark, increased from 0 4 to 0 6% When the solution was stored at 6°C in the dark for about 24 h, the ECTC content increased from 0 4 to 0 9%

Calibration curves and reproducibility

Calibration curves were obtained with the CTC HCl house standard, found to contain 97 7% of CTC HCl and with the reference substances for TC HCl, ECTC HCl, ISOCTC·HCL, ATC·HCl and ACTC HCl found to contain 99 2, 90 9, 98 4, 95 7 and 94 6%, respectively, expressed in terms of the hydrochloride salt The content of DMCTC·HCl (91 2%) was obtained from Cyanamid The determination of the purity of the house standard of CTC and of the reference substances will be discussed elsewhere The following relationships were found, where y = peak area, x = amount (in micrograms) of hydrochloride salt injected, r = correlation coefficient, $S_{y,x}$ = standard error of estimate, CR = range of injected mass examined With mobile phase A CTC, $y = 231 + 8779\,x$, $r = 0\ 9999$, $S_{y,x} = 563$, CR = 20–30 μg, TC, $y = 9023\,x$, $r = 0\ 9997$, $S_{y,x} = 196$, CR = up to 2 5 μg, DMCTC, $y = 7793\,x$, $r = 0\ 9972$, $S_{y,x} = 169$, CR = up to 0 75 μg, ECTC, $y = 5321\,x$, $r = 0\ 9974$, $S_{y,x} = 97$, CR = up to 0 75 μg, ISOCTC, $y = 10332\,x$, $r = 0\ 9933$, $S_{y,x} = 111$, CR = up to 0 25 μg With mobile phase B ATC, $y = 25983\,x$, $r = 0\ 9998$, $S_{y,x} = 206$, CR = up to 1 μg, ACTC, $y = 25186\,x$, $r = 0\ 9986$, $S_{y,x} = 553$, CR = up to 1 μg

The detection limits were 0 05% for TC, ISOCTC, ATC, ACTC, DMCTC and ECTC, and 0 02% for ETC The house standard was analysed 41 times over a period of 6 days The relative standard deviation (RSD) for CTC was 0 4%

Results and Discussion

Development of the chromatographic method

Methods as described for HPLC of TC, doxycycline (DOX) or oxytetracycline (OTC) [11–13] on columns packed with PSDVB and an alkaline mobile phase, cannot be used for the analysis of CTC, since CTC decomposes on the column with formation of ISOCTC Therefore the suitability of classical reversed phases in combination with acid mobile phases such as those formerly used for analysis of TC was further examined [14] It was soon observed that the results could be improved by using perchloric acid, which was also used by Knox *et al* for analysis of TC [15] It is known that in the isocratic analysis of TC with acidic mobile phases, TC and ETC are not sufficiently separated in conditions needed to elute ATC and EATC fast enough to allow detection of small amounts of these impurities [11] This problem is less important in the analysis of CTC Although ACTC and EACTC can be formed by acid decomposition, it was observed

that CTC is more stable than TC towards acid degradation So the determination of anhydroderivatives in CTC samples was of less importance This is also confirmed by the results of the analyses shown below However, it was desirable that for analysis of CTC, a one step gradient method should be available where the second part enabled the determination of the anhydroderivatives Such a one-step gradient method was also used for analysis of OTC [13] and TC [14] When the second part of such a one-step gradient method is considered to be less important due to the small amount of impurities present in commercial samples, the gradient method can be transformed to an isocratic method which enables only the determination of the more important, more polar impurities Instead of a one-step gradient method it is also possible to use a two-step isocratic analysis where the sample is first analysed for the more polar compounds, including the main compound, with a mobile phase containing less organic modifier and where the same sample is later analysed for the less polar compounds with a mobile phase containing more organic modifier This procedure is applicable only when the sample contains very small amounts of less polar impurities, so that during analysis with the less "strong" mobile phase these impurities do not interfere with the following chromatograms

Taking the above mentioned arguments into consideration, attention was first paid to the isocratic separation of ETC, TC, DMCTC, ISOCTC, ECTC and CTC

On a Zorbax C8 column, acetone, acetonitrile, methanol, tetrahydrofuran, t-butanol, dimethylformamide and DMSO were examined as the organic modifier in mobile phases which further contained 5% of 1 M perchloric acid and water The best results were obtained with DMSO that gave more symmetrical peaks and good separation of ECTC and CTC

In order to examine the general applicability of this organic modifier, a mixture of reference substances was chromatographed on a series of columns listed in Table 1 The amount of organic modifier was adjusted to obtain a capacity factor (k') of 6–9 for CTC To achieve this, mobile phases containing 5–55% of DMSO had to be used All the experiments were carried out at 35°C The results (k' values) are shown in Fig 2 Except for ISOCTC, the elution order was the same on all the columns On most reversed-phase columns, ISOCTC was eluted before, with or after ECTC, and on columns V and XI after CTC On the PSDVB column XX, ISOCTC was even eluted before DMCTC Very poor retention was obtained on cyano column XIX which also gave the poorest separation It is therefore concluded that cyano columns are not suitable for analysis of CTC with this mobile phase The selectivity of different C8 and C18 columns towards the impurities was somewhat variable but except for ISOCTC on certain columns, it was possible to separate the main component CTC from all the impurities on all the columns examined This is the most important characteristic of a good method for quantitative determination of the antibiotic It will be seen below that ISOCTC is only a minor impurity and therefore coelution of ISOCTC with CTC as observed on some of the columns or with another impurity can be considered as a minor disadvantage of the method

It is also observed in Fig 2 that different columns packed with the same brand of reversed-phase material can give different retention (see Table 1 for the DMSO content in the mobile phase) and small differences in selectivity, especially for ISOCTC For Zorbax C8 it was observed that on the much older and more intensively used column I, the retention was weaker than on columns II, III and IV The order of elution on column I was also different Columns II, III and IV showed only small differences in selectivity,

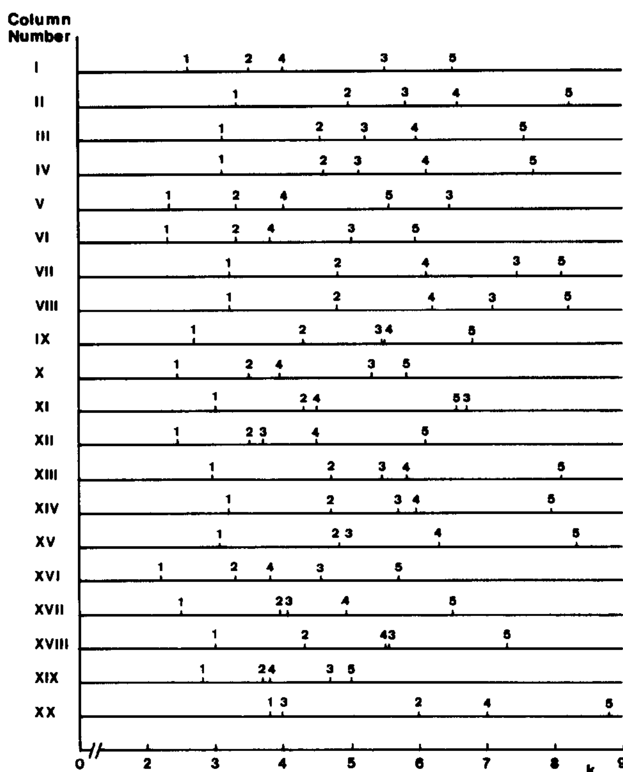

Figure 2
Capacity factors of chlortetracyclıne and related substances on columns I–XX See Table 1 for column ıdentıfıcatıon Mobıle phase DMSO–1 M perchlonc acıd–water [x 5 (95 $-x$)], x ıs specıfied ın Table 1 Flow rate, 1 ml mın^{-1}, temperature, 35°C, detectıon, UV at 280 nm Substance ıdentıfıcatıon 1, TC, 2, DMCTC, 3, ISOCTC, 4, ECTC, 5, CTC

the latter two beıng packed wıth the same batch of reversed-phase materıal Thıs phenomenon can be partly due to batch-to-batch dıfferences but ıs belıeved to be maınly dependent upon column age and hıstory Thıs was also concluded ın a recent study concernıng HPLC of erythromycın [16]

It can be concluded that many reversed-phase materıals can be used for quantıtatıve determınatıon of CTC wıth the mobıle phase descrıbed Further work ın thıs laboratory was carrıed out on Zorbax C8

Fıgure 3 shows the ınfluence of the concentratıon of DMSO ın the mobıle phase on the separatıon of CTC and related substances at 35°C It was observed that dependıng upon the DMSO content, the relatıve posıtıon of ISOCTC was shıfted Fıgure 4 shows the ınfluence of temperature A temperature of 35°C was retaıned sınce ıt allowed good separatıon of ISOCTC and ECTC and ıt ımplıed slıght heatıng throughout the year The ınfluence of the concentratıon of perchlorıc acıd ın the mobıle phase was brıefly ınvestıgated A concentratıon of 1% of 1 M perchlorıc acıd gave a much better separatıon than 0 2% Further ıncrease of the concentratıon of 1 M perchlorıc acıd up to 10%, ımproved the separatıon only slıghtly An ıntermedıate concentratıon of 5% 1 M perchlorıc acıd was retaıned It was observed that wıth a mıxture of 1% 1 M perchlorıc

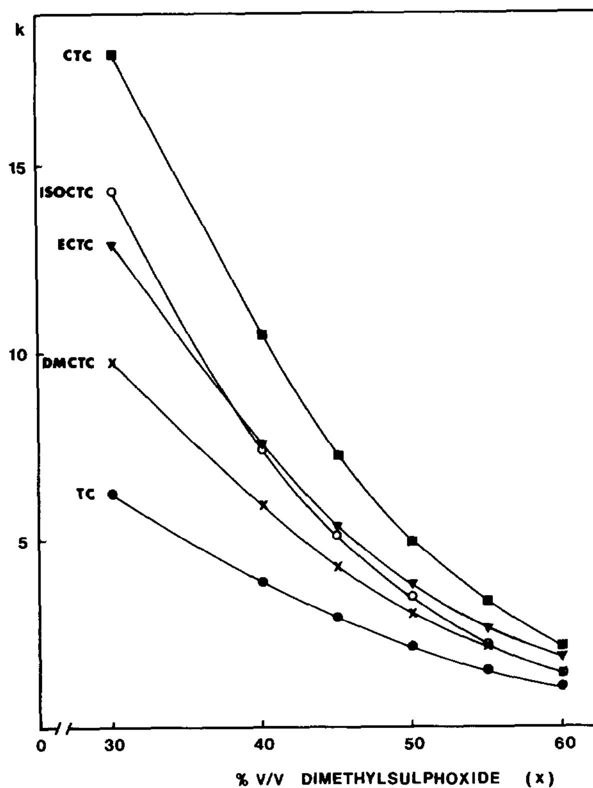

Figure 3
Influence of the concentration of organic modifier in the mobile phase on the separation of CTC and related substances Column Zorbax C8 7 μm, 250 × 4 6 mm Mobile phase DMSO–1 M perchloric acid–water [x 5 (95 − x)] Flow rate, 1 ml min^{-1}, temperature, 35°C, detection, UV at 280 nm

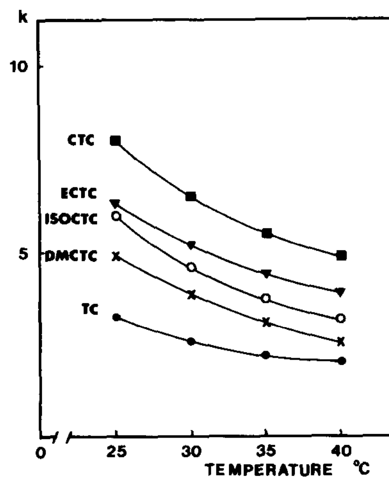

Figure 4
Influence of temperature on the separation of CTC and related substances Column Zorbax C8 7 μm, 250 × 4 6 mm Mobile phase DMSO–1 M perchloric acid–water (50 5 45) Flow rate, 1 ml min^{-1}, detection, UV at 280 nm

acid and 4% 1 M sodium perchlorate, the separation was less good, which indicates that not only the perchlorate concentration, but also the total acidity plays a role

Figure 5 shows a chromatogram obtained by isocratic elution of a spiked CTC sample It is clear that a CTC sample cannot be analysed for all the related substances by one isocratic method Because gradient elution with DMSO as the organic modifier and UV detection at 280 nm gives a considerable shift of the baseline, it was decided to use two isocratic methods for analysis of CTC samples The result obtained for a commercial sample is shown in Fig 6 Mobile phase A allows the quantitation of CTC, and the more polar related substances and mobile phase B allows the quantitation of the less polar related substances It appears that small amounts of ETC and ATC can be present in commercial samples Figures 5 (55% DMSO) and 6A (32% DMSO) are also an example of the shift of the position of ISOCTC in function of the DMSO content of the mobile phase During analysis of DOX, OTC and TC on PSDVB stationary phase using alkaline mobile phase, 2-acetyl-2-decarboxamido-derivatives were separated right after the main

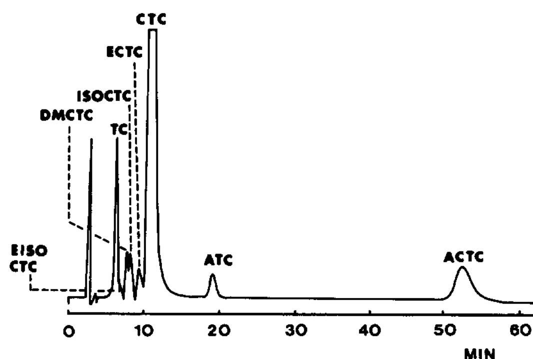

Figure 5
Isocratic separation of a spiked sample of CTC Column Zorbax C8 7 μm, 250 × 4 6 mm Mobile phase DMSO–1 M perchloric acid–water (55 5 40) Flow rate, 1 ml min^{-1}, temperature, 35°C, detection, UV at 280 nm

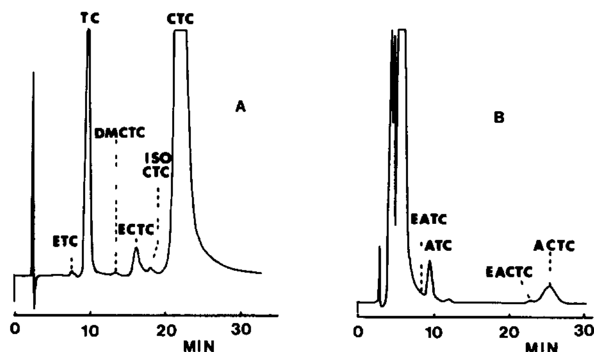

Figure 6
Chromatogram of a typical commercial sample of chlortetracycline obtained by (A) slow isocratic elution, (B) fast isocratic elution Column Zorbax C8 7 μm, 250 × 4 6 mm Mobile phase DMSO–1 M perchloric acid–water [x 5 (95 − x)], Flow rate, 1 ml min^{-1}, temperature, 35°C, detection, UV at 280 nm A, slow isocratic elution, x = 32, B, fast isocratic elution, x = 60

component [12, 13, 17] 2-Acetyl-2-decarboxamidochlortetracycline (ADCTC) is probably not separated from CTC by the method described above Since ADCTC was not available, it was not possible to check this separation but it was shown that the corresponding ADTC and TC were not separated with a mobile phase where the DMSO content was decreased to obtain the same retention for TC as that normally obtained for CTC

For the analysis of official standards and samples mentioned below, two isocratic methods were used with mobile phases A and B already mentioned above When analyses were performed with mobile phase A over a period of several days, it was observed that the retention time for CTC slowly decreased This was also observed for stationary phases other than Zorbax C8 Although it did not affect the separation over a short period, the composition of the mobile phase had to be slightly adapted after about 2 weeks by decreasing the DMSO content by a few percent Over longer periods this improved the separation of ECTC and ISOCTC since the relative retention of ISOCTC depended upon the DMSO content as shown in Fig 3 It can be concluded that the separation of ECTC and ISOCTC on Zorbax C8 improves as the column material deteriorates In our laboratory it had been previously observed for analysis of erythromycin, that older columns gave better separations [16]

Comparison of standards

The CTC HCl house standard was titrated with perchloric acid in non-aqueous conditions Two independent series of titrations gave a mean of 99 1% CTC HCl (RSD = 0 3%) for a total number of 18 titrations Two independent series of Karl Fischer titrations gave a mean of 0 9% (RSD = 5 5%) for a total number of 15 titrations This result was confirmed by loss on drying over P_2O_5 under vacuum for 5 h (see Table 2) It was observed that a drying period of 3 h as prescribed by the USP and by the Ph Eur [18, 19], was insufficient and that it should be prolonged to 5 h The total content of the CTC HCl house standard was therefore accepted to be 99 1%, 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 1 4% So the CTC HCl house standard was assigned a purity of 97 7%

Using the CTC HCl house standard, the content of the official standards was determined by HPLC The CTC HCl content was determined on several days by comparison with the chromatograms obtained for the CTC HCl house standard on the same days The impurities were determined as mentioned above for the CTC HCl house standard Table 2 summarizes the results obtained The RSD values given in parentheses below the content are within acceptable limits for all the determinations Of all standards, the USP-RS H-1 contained most TC (1 3%), the WHO-IS contained most ECTC (0 6%) and the house standard contained most DMCTC (0 4%) The other impurities were of minor importance Non-aqueous titrations, Karl Fischer titrations and loss on drying, were not carried out for the official standards, owing to the limited amount available Some values for loss on drying were obtained from elsewhere [20–22] The amount of water and volatile substances did not contribute much to the total mass of the standards The total mass explained by the analytical values available is always very close to 100% The Ph Eur -CRS (1000 i u mg^{-1}) and the WHO-IS (1000 i u mg^{-1}), which have the same declared biological activity, have a comparable CTC content The slightly higher activity claimed for Ital -RS compares well with the somewhat higher content The USP-RS H-1 (1000 $\mu g\ mg^{-1}$) has a somewhat lower biological activity than

Table 2
Composition of chlortetracycline standards

	House standard	Ph Eur -CRS 1000 i u mg^{-1}	USP-RS H-1 1000 µg mg^{-1}	USP-RS I-1 1007 µg mg^{-1}	WHO-IS (second) 1000 i u mg^{-1}	Ital -RS 1007 i u mg^{-1}
Number of solutions	36	11	6	7	5	5
Number of analyses	41	19	12	16	14	13
Number of days	6	6	5	6	5	5
ETC*	0 02 (31)	<0 02	0 05 (26)	<0 02	<0 02	<0 02
TC	0 4 (6 3)	0 2 (27)	1 3 (3 5)	<0 05	0 2 (5 2)	0 4 (2 0)
DMCTC	0 4 (13)	0 05 (46)	0 2 (28)	<0 06	0 2 (53)	0 07 (35)
ECTC	0 5 (14)	0 2 (20)	0 2 (31)	0 2 (12)	0 6 (13)	<0 05
ISOCTC	<0 05	<0 05	<0 05	<0 05	0 2 (2 0)	0 06 (39)
ACTC‖	0 1 (1 1)	<0 05	<0 05	0 1 (4 7)	<0 05	<0 05
CTC	97 7 (0 4)	98 5 (0 2)	97 0 (0 3)	98 9 (0 4)	98 2 (0 3)	98 8 (0 3)
Subtotal	99 1	99 0	98 8	99 2	99 5	99 3
Titration	99 1	ND	ND	ND	ND	ND
n (RSD)	18(0 3)					
Water determined†	0 9	ND	ND	ND	ND	ND
n (RSD)	15(5 5)					
Loss on drying determined *n* (RSD)	0 7§	ND	ND	ND	ND	ND
Loss on drying declared		0 9‡ [20]	NA	0 8‡ [21]	0 3‡ [22]	NA

Values in percent (m/m) expressed in terms of the hydrochloride, n = number of analyses, RSD values are given in parentheses * Expressed in terms of TC HCl † Karl Fischer ‡ At 60°C for 3 h over P_2O_5 under vacuum § At 60°C for 5 h over P_2O_5 under vacuum ‖ Determined with fast isocratic elution, three analyses of one solution (mobile phase B) ATC is below the detection limit (0 05%), NA = not available, ND = not determined owing to the limited amount of sample

the USP-RS I-1 (1007 μg mg^{-1}) which is confirmed by the CTC content If the micrograms of the USP standards are interpreted as mass units, then the declared activity is an overestimate since none of the USP standards has a purity of 100% In fact, the micrograms reported here have to be interpreted as micrograms of activity This can be a source of confusion [23]

Analysis of commercial samples

Commercial samples were analysed as described above for standards Table 3 shows the results for CTC·HCl bulk samples For simplicity, RSD values are not mentioned for the impurities The reproducibility of the CTC assay is good The samples contain up to 7 9% of TC, up to 3 7% of ECTC and up to 1 2% of DMCTC ISOCTC is present up to 0 4% For samples containing more than 1% of ECTC, amounts of ISOCTC smaller than 0 2% were not sufficiently separated from ECTC, and were not integrated separately ATC is present up to 0 2% and ACTC up to 0 3% The ratio ATC/TC in the samples being greater than the ratio ACTC/CTC, is an indication for CTC being more stable than TC towards acid degradation All the samples contained small amounts of ETC EACTC, EATC and DMTC were never present at levels above 0 05% DMTC is eluted between ETC and TC Unlike for TC, the Ph Eur and USP do not limit the amount of impurities, although CTC samples appear to be less pure than TC samples Samples containing high amounts of impurities are of course excluded by determination of the potency by microbiological assay Under the conditions of this assay, TC is less active than CTC However, the TC content of CTC samples should be limited The water content was within Ph Eur and USP limits (2%) Owing to the rather limited amount of sample available, Karl Fischer titrations were carried out instead of loss on drying which is prescribed by the official texts For most samples, the total content was close to 100% Samples 2, 8 and 9 had a distinctly lower content The former two were known to be more than 15 years old, but the relative amounts of impurities were comparable to those of the other samples It is believed that the low content of these samples was rather due to impurities which were not detected by this HPLC method and which were present in the samples since the time of preparation The total content of sample 8 (96 5%) corresponded well with the label content (96 2%), claimed to be obtained by chemical assay Like OTC HCl [13] and TC HCl [14], solid CTC HCl is very stable when stored in dry conditions This was not investigated in detail as was done for OTC HCl and TC HCl, but it was observed that a dried sample was not significantly decomposed by heating at 70°C for 6 years and only small amounts of ATC and ACTC were formed

Table 4 gives the composition of a number of preparations The relative amounts of impurities were comparable to those found for the CTC HCl bulk samples except for sample 22 which contained 8 3% of ECTC Other, much older preparations contained far less ECTC Most samples had a CTC HCl content above 95% of the label claim Sample 25 had a very low content (81 5%) This sample also contained sulphanilamide which coeluted with ISOCTC Therefore no figure is mentioned for ISOCTC All other samples complied with the 90–120% USP limits for capsules or tablets or with the 90–125% USP limits for ointments

Conclusion

The results obtained have shown that the HPLC method described is very suitable for quantitative analysis of chlortetracycline in bulk samples and in preparations

Table 3
Composition of bulk samples of chlortetracycline hydrochloride

Sample origin	Sample number	Number of solutions analysed	CTC: Number of analyses	CTC: Mean (%)	CTC: RSD (%)	ETC*	TC	DMCTC	ECTC	ISOCTC	ATC†	ACTC†	Water content (Karl Fischer): Number of analyses	Water content (Karl Fischer): Mean (%)	Water content (Karl Fischer): RSD (%)	Total (%)
Manufacturer																
A	2	3	3	89.7	0.2	0.02	3.4	0.4	0.7	0.2	<0.05	0.07	3	0.7	1.5	95.2
	3	3	3	94.3	0.2	<0.02	4.7	0.2	1.0	‡	0.08	0.07	3	0.4	2.7	100.8
	4	3	3	93.7	0.3	0.02	4.8	0.2	1.0	‡	<0.05	0.09	3	0.6	5.1	100.4
	5	3	3	94.2	0.2	0.02	4.5	0.3	1.3	‡	0.08	0.07	3	0.3	9.1	100.8
	14	3	3	89.1	0.2	<0.02	7.1	0.2	0.4	<0.05	<0.05	<0.05	3	1.8	1.6	98.8
	15	3	3	91.5	0.5	<0.02	5.8	0.5	0.4	<0.05	<0.05	<0.05	3	1.1	4.3	99.3
B	8	3	3	88.7	0.5	0.03	4.8	0.09	1.7	0.4	0.2	0.09	3	0.5	4.2	96.5
Wholesalers																
1	6	3	3	91.7	0.5	0.03	3.7	0.4	3.7	‡	0.1	0.3	3	0.4	1.3	100.3
	7	4	4	87.8	0.4	0.1	7.5	0.08	3.0	0.3	<0.05	0.08	3	0.8	8.5	99.7
	11	3	3	91.9	0.4	0.03	4.6	0.2	2.9	‡	0.2	0.3	3	0.5	5.0	100.6
2	1	3	3	92.0	0.3	0.03	4.7	1.2	2.1	‡	0.2	0.2	3	0.5	6.0	100.9
	9	3	3	89.1	0.2	0.02	4.6	1.1	2.0	‡	0.2	<0.05	3	0.6	1.0	97.6
	12	4	4	89.1	0.5	0.1	7.9	0.06	2.2	0.2	0.2	<0.05	3	0.8	0.7	100.6
3	10	3	3	91.1	0.6	0.03	4.3	1.0	2.7	‡	0.2	0.2	3	0.5	3.7	100.0
4	13	3	3	89.5	0.3	0.06	7.1	0.08	1.3	0.2	<0.05	<0.05	3	0.8	3.4	99.0

Values in percent (m/m) expressed in terms of hydrochloride. * Expressed in terms of TC HCl. † Obtained by one analysis with fast isocratic elution (mobile phase B). ‡ Small amount present, not integrated separately but calculated together with ECTC. Sample age is not mentioned on label.

Table 4
Composition of specialties as a percentage (m/m) of label claim

Manufacturer	Sample number	Sample form*	Sample age in months	Number of solutions analysed	Number of analyses	ECT†	TC	DMCTC	ECTC	ISOCTC	ATC‡	ACTC‡	CTC Mean (%)	CTC RSD (%)
A	16	Ointment	26	3	3	0 02	4 7	0 2	1 1	§	<0 05	0 05	95 6	0 7
	17	Ointment	36	3	3	0 02	4 9	0 2	1 1	§	<0 05	0 05	96 3	0 4
	18	Ointment	42	4	4	<0 02	4 8	0 2	1 1	§	<0 05	<0 05	96 8	0 4
	19	Ointment	14	2	5	0 03	4 7	0 3	1 6	§	<0 05	<0 05	96 8	0 7
	20	Capsules	60	3	3	0 1	7 1	0 3	3 6	§	<0 05	<0 05	101 3	0 4
	21	Capsules	80	3	3	0 1	7 6	0 2	4 7	§	<0 05	<0 05	92 3	0 6
C	22	Veterinary gynaecological tablets	17	3	3	0 2	3 9	0 4	8 3	§	0 08	0 1	89 8	0 5
	23	Veterinery gynaecological suppositories	60	3	3	0 2	9 6	0 07	3 0	0 3	<0 05	<0 05	94 8	0 3
D	24	Veterinary local spray	42	1	4	0 04	4 7	0 6	3 9	§	0 06	0 1	98 6	0 5
E	25	Veterinary local spray	14	1	4	0 2	6 6	0 3	3 1	ND	<0 05	<0 05	81 5	0 1

Values in percent (m/m) expressed in terms of the hydrochloride * Chlortetracycline is present as the hydrochloride † Expressed in terms of TC HCl ‡ Obtained by one analysis with fast isocratic elution (mobile phase B) § Small amount present, not integrated separately but calculated together with ECTC ND = not detected

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