Pergamon

0731-7085(94)00105-7

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 12, pp. 1483–1488, 1994

0731-7085/94 $7.00 + 0.00

Evaluation of tetracycline raw materials and finished products found on the Kenyan market

JOHN WAITITU MURITU, ISAAC ONGUBO KIBWAGE,* CHARLES KARIMI MAITAI and JOS HOOGMARTENS†

Department of Pharmacy, College of Health Sciences, University of Nairobi, P.O. Box 19676, Nairobi, Kenya
† Laboratorium voor Farmaceutische Chemie, Instituut voor Farmaceutische Wetenschappen, Van Evenstraat 4, 3000 Leuven, Belgium

Abstract: Contents of tetracycline, its degradation products (epitetracycline, epianhydrotetracycline, anhydrotetracycline) and a fermentation impurity (2-acetyl-2-decarboxamidotetracycline) were determined in four raw materials, 12 batches of six ointment products, four eye ointment products and nine batches of five capsule products, all sampled from the Kenyan market. The analytical method was liquid chromatography on a column packed with a poly(styrenedivinylbenzene) material (8-μm PLRP-S 100 Å). All raw materials and finished products had tetracycline contents and impurity levels within the prescribed compendial limits.

Keywords: *Tetracycline; impurities; raw materials; finished products.*

Introduction

Tetracycline (TC) is a member of the tetracycline family which is one of the most important groups of broad-spectrum antibiotics. TC is active against many Gram-negative and Gram-positive bacteria as well as rickettsiae. TC was first prepared by the catalytic reduction of chlortetracycline [1] but is currently produced by many strains of *Streptomyces aureofaciens*, *Streptomyces avellanus*, *Streptomyces feofaciens*, *Streptomyces alboflavus* and many others [2]. Of the tetracycline family TC is the most widely used in therapeutics.

At pH 2.0–6.0, TC undergoes reversible epimerization at C-4 to form an equilibrium mixture of 4-epitetracycline (ETC) and tetracycline. Epimerization strongly reduces the antibiotic activity of tetracycline to 2–5% [3]. Owing to the presence of a tertiary hydroxyl group at C-6 in tetracycline, acid degradation takes place resulting in the formation of anhydrotetracycline (ATC). ATC also undergoes epimerization at C-4, resulting in the formation of 4-epianhydrotetracycline (EATC). EATC can also be formed by acid degradation of ETC [3, 4]. Another related compound, 2-acetyl-2-decarboxamidotetracycline (ADTC) has been described [5] and was later mentioned as a fermentation impurity of TC [6, 7]. ADTC is practically inactive therapeutically.

EATC shows a definite toxicity to renal tubular function and initiates a reversible Fanconi-type syndrome characterized by polyuria, polydyspepsia, vomiting, proteinuria, glycosuria, acidosis and aminoaciduria [8–10].

All these degradation and fermentation products of TC are present as impurities in varying amounts in raw materials and finished products of TC. Owing to the toxicity and/or therapeutic inactivity of these degradation and fermentation products it is very important that their contents in TC products be controlled.

In Kenya, TC is presented as capsules, ointments and eye ointments. These formulations are manufactured locally using raw materials obtained on the international market. The locally manufactured products are supplemented by imported brands.

There have been allegations in the mass media of dumping of finished products and raw materials of poor quality and of the local manufacture of substandard products. Such allegations if true could result in widespread resistance to use of the drug and the Govern-

* Author to whom correspondence should be addressed.

ment would then be forced to divert resources to the purchase of alternative and expensive medicines to maintain the health of the nation. In addition, since Kenya is an exporter of pharmaceuticals to the eastern, central and southern regions of Africa, drugs originating from Kenya would be brought into disrepute.

The objective of this study was to analyse the content and composition of TC and related impurities in TC raw materials and finished products found on the Kenyan market.

It has been shown that with liquid chromatography (LC), using slightly alkaline mobile phases, very good separations of TC and all its degradation and fermentation products can be obtained with poly(styrene-divinylbenzene) packing materials [11]. This method has subsequently been adopted by the European Pharmacopoeia (Ph. Eur.) for the assay of TC preparations [12]. In this study, done at the Department of Pharmacy, University of Nairobi, analyses were performed on a column packed with 8-μm PLRP-S 100 Å.

Materials and Methods

Reference samples

The reference samples of TC.HCl, ETC.HCl, ATC.HCl and EATC.HCl were obtained from Laboratorium voor Farmaceutische Chemie, Katholieke Universiteit Leuven, Belgium. Owing to the limited amount of the TC.HCl reference sample, the composition of a working standard was determined by LC and non-aqueous titration and found to contain TC.HCl 97.9% and ADTC 0.7%, expressed as TC. A pure reference substance of ADTC was not available.

Solvents and reagents

2-Methyl-2-propanol was from Janssen Chimica (Beerse, Belgium) and was distilled before use. Other analytical grade reagents, tetrabutylammonium hydrogen sulphate (TBA), disodium ethylenediaminetetraacetate (EDTA) were also from Janssen Chimica. Disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate were from BDH Chemicals (Poole, UK). Water was distilled from a glass apparatus.

Method and operating conditions

Isocratic elution was used throughout the study. The LC apparatus comprised a Merck–Hitachi L-6200 Intelligent pump (Darmstadt, Germany), a Valco injector model CV-6-UHPa-N60 (Houston, TX, USA) equipped with a 25-μl loop, a Merck–Hitachi L-4200 UV-visible detector set at 254 nm and a Hewlett–Packard 3396 Series II integrating recorder (Avondale, PA, USA). A 250 × 4.6 mm i.d. column packed with 8-μm PLRP-S 100 Å (Polymer Labs, Church Stretton, Shropshire, UK) was immersed in a water-bath heated at 60°C. The flow-rate of the mobile phase was 1.0 ml min^{-1}.

Mobile phase

The mobile phase was 2-methyl-2-propanol–sodium phosphate buffer (pH 9.0; 0.2 M)–TBA (pH 9.0; 0.02 M)–EDTA (pH 9.0; 0.01 M) (7.05:10:15:10, m/v/v/v). This mixture was diluted with water to 100 parts by volume. During the preparation of the phosphate buffer, TBA and EDTA solutions, the pH was adjusted using 5 M NaOH. The mobile phase was degassed by ultrasonication.

Sampling

Tetracycline capsules and ointments were sampled from pharmacies in Nairobi. Others were samples submitted to the Pharmacy and Poisons Board for registration. Sample raw materials were obtained from manufacturers of finished products. In the text, product and manufacturer are used interchangeably. Nairobi was chosen because it is a microcosm of Kenya. All pharmaceutical manufacturers and distributors are also situated within Nairobi.

Sample preparation

Ointments. TC ointment equivalent to 50 mg TC.HCl was transferred using *n*-hexane into a 50-ml separating funnel. Ten millilitres of 0.01 M HCl was added and the mixture was shaken. The lower aqueous layer which contained the extracted tetracyclines was collected into a 50-ml volumetric flask. The extraction was repeated using three additional 10-ml portions of 0.01 M HCl and the extracts were combined in the 50-ml volumetric flask. The extract was diluted to volume with 0.01 M HCl, thoroughly mixed, filtered through a 0.45-μm membrane filter and injected into the chromatograph.

Capsules. Twenty capsules were accurately weighed and the contents emptied into a mortar. The empty capsule shells were cleaned

and weighed. The net weight of the capsules was calculated. The powder was mixed using a pestle and an amount equivalent to 25 mg TC.HCl was weighed into a 25-ml volumetric flask. Of HCl, 0.01 M was then added, the mixture sonicated, filtered through a 0.45-μm membrane filter and injected into the chromatograph.

Results and Discussion

The liquid chromatographic method used to analyse TC preparations was found to be easily applicable. The quality of separations obtained also remained unchanged for the duration of the study. Figure 1 shows a typical chromatogram.

Calibration curves and repeatability

Seven-point calibration curves were obtained with the TC.HCl working standard. Each point was a mean of three independent analyses. The TC.HCl working standard had a content of 97.9% TC.HCl. The contents of the reference substances ETC.HCl, EATC.HCl and ATC.HCl were 84.5, 94.3 and 91.0%, respectively, all expressed in terms of the hydrochloride salt. The following relationships were found, where Y = peak area, X = amount of hydrochloride salt injected, and CR = range of injected mass examined. For TC.HCl: CR = 9–40 μg; $Y = 3074447X - 3913410$; $r = 0.9999$; $S_{y,x} = 1646131$. For ETC.HCl: CR = 0.2–2.3 μg; $Y = 3882169X + 257852$; $r = 0.9998$; $S_{y,x} = 51749$. For ATC.HCl: CR = up to 3 μg; $Y = 6676849X - 135365$; $r = 0.9994$; $S_{y,x} = 306155$. For EATC.HCl: CR = up to 0.95 μg; $Y = 6013580X - 42681$; $r = 0.9999$; $S_{y,x} = 15239$. The repeatability of the chromatographic system was checked by analysing the TC working standard 28 times over a period of 4 days. The relative standard deviation (RSD) for TC was 0.8%.

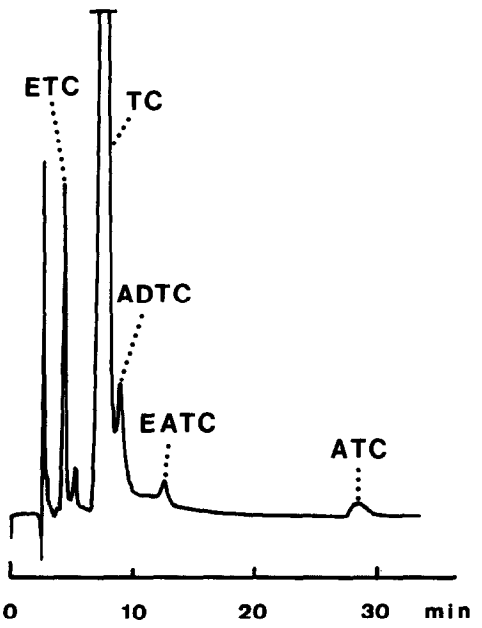

Figure 1
A typical chromatogram of a sample from tetracycline capsules. Peaks: 1 = 4-epitetracycline (ETC); 2 = tetracycline (TC); 3 = 2-acetyl-2-decarboxamidotetracycline (ADTC); 4 = 4-epianhydrotetracycline (EATC); 5 = anhydrotetracycline (ATC). Chromatographic conditions are as described in the text.

Analysis of samples

The percentage content of ETC.HCl, EATC.HCl and ATC.HCl in the samples was calculated using the calibration curve for each impurity. ADTC was calculated in terms of TC.HCl because no pure reference sample was available. For this purpose, the peak area ratio ADTC/TC in each chromatogram was used. The UV spectra of TC and ADTC are similar [7]. TC was calculated by comparing the area of the TC peak of the sample with that of the TC peak of the TC working standard obtained the same day.

Analysis of tetracycline raw materials

Four lots of raw materials were obtained from three local manufacturers of TC products and analysed. The results are shown in Table 1. The RSD for TC.HCl is less than 0.25%. This shows that the repeatability for TC.HCl is very good. The samples contain 0.8–1.2% ETC.HCl, 0.6–0.8% ADTC.HCl, 0.08–0.27% EATC.HCl and 0.13–0.28% ATC.HCl. The Ph.Eur. prescribes limits of 5% for ETC and 0.5% for EATC and ATC in tetracycline raw materials [12]. The United States Pharmacopeia (USP, 1990) limits the amount of EATC in tetracycline bulk samples to 2% [13]. Thus from Table 1, the contents of ETC.HCl, EATC.HCl and ATC.HCl are all below the limits prescribed by the Ph.Eur. and the USP. The moisture content of the raw materials as determined by Karl Fischer titration was found to be 1.12–1.89%, which lies within the 2% limits prescribed by the Ph.Eur. and USP monographs. The TC.HCl present in all samples examined was more than 95%. The USP prescribes that the potency of TC.HCl in

bulky (raw) materials should not be less than 900 $\mu g\ mg^{-1}$. Thus the TC.HCl content of the raw materials complies with the limits set by the USP.

Analysis of tetracycline ointments

Commercial samples of tetracycline ointments were analysed as for the raw materials. Table 2 shows the analytical results for six ointments. There were five batches of product A and two each of products B and G. Table 3 shows the results for four eye (ophthalmic) ointments. The TC.HCl content of the ointments and eye ointments was between 90 and 112%. The USP (1990) prescribed limits for TC.HCl content in ointments are 90–125%. Thus the TC.HCl content of the ointments examined is well within the limits prescribed by the USP. The TC content of 92.03, 90.2 and 91.24%, respectively, for products E and F,

Table 1
Composition* of some raw materials of tetracycline hydrochloride†

	TC		Mean (%)					
Manufacturer‡	Mean (%)	RSD (%)	ETC	ADTC	EATC	ATC	Water	Total
A	96.95	0.21	0.86	0.63	0.12	0.28	1.15	99.99
A	96.92	0.14	1.20	0.80	0.27	0.27	1.12	100.58
B	95.93	0.19	1.08	0.67	0.08	0.26	1.21	99.23
C	95.42	0.23	1.28	0.82	0.41	0.13	1.89	99.95

*Composition (%, w/w) is expressed in terms of the hydrochloride; each value is the mean of three independent analyses.
†Sample age not stated.
‡Local user of raw material.

Table 2
Composition* of tetracycline ointments as a percentage (w/w) of the label claim

		TC					
Product	Sample age†	Mean (%)	RSD (%)	ETC (%)	ADTC (%)	EATC (%)	ATC (%)
A	3	101.38	0.52	2.03	1.15	0.24	0.62
A	3	99.93	0.21	1.94	1.01	0.15	0.49
A	9	101.23	0.32	1.36	1.08	0.16	0.42
A	10	101.32	0.61	2.20	1.16	0.14	0.52
A	10	91.24	0.30	1.39	1.33	0.13	0.40
B	NS‡	107.66	0.27	1.24	1.06	0.25	0.43
B	1	99.89	0.32	1.27	1.17	0.09	0.39
D	7	94.35	0.38	1.10	1.04	0.22	0.84
E	7	92.03	10.80	1.41	0.90	0.14	0.40
F	11	90.20	0.41	1.57	0.11	0.13	0.43
G	5	98.15	4.25	0.83	1.28	0.08	0.45
G	8	106.22	0.60	1.45	1.20	0.06	0.42

*Composition expressed in terms of the hydrochloride, each value is the mean of three independent analyses.
†Age in months.
‡Not stated.

Table 3
Composition* of tetracycline eye ointments as a percentage (w/w) of the label claim

		TC					
Product	Sample age†	Mean (%)	RSD (%)	ETC (%)	ADTC (%)	EATC (%)	ATC (%)
H	29	111.74	1.81	1.71	0.46	0.94	0.99
J	18	99.32	16.77	0.83	0.89	0.12	0.33
D	13	104.23	1.75	1.34	1.36	0.15	0.46
K	11	104.82	4.36	1.89	0.68	0.07	0.62

*Composition expressed in terms of the hydrochloride; each value is the mean of three independent analyses.
†Age in months.

Table 4
Composition* of tetracycline capsules as a percentage (w/w) of the label claim

		TC						Water	
Product	Sample age†	Mean (%)	RSD (%)	ETC (%)	ADTC (%)	EATC (%)	ATC (%)	Mean (%)	RSD (%)
A	1	90.22	0.78	0.83	0.60	0.10	0.25	1.41	0.02
B	1	101.10	1.23	1.12	0.66	0.08	0.26	1.41	0.68
D	17	104.38	0.34	1.36	1.27	0.35	0.86	1.16	0.24
D	28	103.40	1.45	2.11	1.26	0.39	0.94	1.45	1.21
D	28	103.40	1.30	2.15	1.21	0.36	0.94	1.26	0.86
L	12	98.82	0.79	0.70	0.70	0.39	0.47	1.49	0.12
L	20	97.90	0.42	1.03	0.63	0.18	0.26	1.32	0.42
L	20	97.02	0.42	1.11	0.82	0.16	0.25	1.34	0.23
M	11	100.71	0.21	1.71	0.82	0.09	0.39	1.33	0.43

*Composition expressed in terms of the hydrochloride; each value is the mean of three independent analyses.
†Age in months.

and one batch of A, was close to the lower limits. The ointments contained 0.11–1.4% ETC.HCl, 0.06–0.95% EATC.HCl and 0.3–1.0% ATC.HCl. The USP limits the amount of EATC.HCl in preparations to 3%. The EATC.HCl content of the ointments was much lower than 3%. Even the USP limit of 2% EATC.HCl and the Ph.Eur. limit of 5% ETC.HCl in TC raw materials were never reached in the ointments examined. The variable TC content of batches of product A may perhaps be due to poor adherence to good manufacturing practice by the manufacturer.

Analysis of tetracycline capsules

The analytical results for capsules are shown in Table 4. There were nine batches from five products. The TC.HCl content of the capsules ranged from 90 to 105% of the label claim. The USP (1990) limits TC.HCl in capsules to 90–125.0%. Thus the TC.HCl content of all the capsules was within the limits prescribed by the USP. The ETC.HCl content was 0.7–2.2%, ADTC.HCl was 0.6–1.3%, EATC.HCl was 0.08–0.4% and ATC.HCl was 0.25–1.0%. The EATC.HCl content was much lower than the 3% maximum limit set by the USP. The ETC.HCl and EATC.HCl contents were also lower than the limits of 5 and 0.5%, respectively, set by the Ph.Eur. (1983) for bulk TC.HCl. The water content of the capsules ranged from 1.2 to 1.5%. This is within the USP maximum limit of 4% and less than the 3% loss on drying prescribed by the British Pharmacopoeia (1988) [14]. The RSD values for water content were less than 1.5%. This is an indication of good repeatability of water determination.

The results in Tables 2–4 suggest that the age of the ointment or capsule does not seem to affect the content of tetracycline and/or tetracycline degradation products. Old samples have the same content and composition as fresh samples. In general, the tetracycline raw materials and finished products found on the Kenyan market had contents of TC and degradation products that were within the limits set by pharmacopoeias.

Acknowledgements — The authors thank the Belgian Administration for Development Cooperation for financial support and Mr J.K. Thuranira for technical assistance.

References

[1] J.H. Boothe, J.H. Morton, J.P. Petisi, R.G. Wilkinson and J.H. Williams, *J. Am. Chem. Soc.* **75**, 4621 (1953).
[2] M.J. Weinstein and G.H. Wagman, *Antibiotics, Isolation, Separation and Purification*. Journal of Chromatography Library, Vol. 15. Elsevier, Amsterdam (1978).
[3] L.A. Mitscher, *The Chemistry of the Tetracycline Antibiotics*. Medicinal Research Series, Volume 9. Marcel Dekker, New York (1978).
[4] J.R.D. McCormick, S.M. Fox, L.L. Smith, B.A. Bitler, J. Reichenthal, V.E. Origoni, W.H. Muller, R. Winterbottom and A.P. Doerschuk, *J. Am. Chem. Soc.* **79**, 2849–2858 (1957).
[5] M.W. Miller and F.A. Hochstein, *J. Org. Chem.* **27**, 2525–2528 (1962).
[6] I. Keiner, R. Huettenrauch and W. Poethke, *Arch. Pharm.* **300**, 840–844 (1967).
[7] G.C. Lancini and P. Sensi, *Experientia* **20**, 83–84 (1964).
[8] G.W. Frimpter, A.E. Timpanelli, W.J. Eisenmenger, H. Stern and L.Z. Ehrlich, *J. Am. Med. Assoc.* **184**, 111–113 (1963).
[9] J.M. Gross, *Ann. Int. Med.* **58**, 523–528 (1963).
[10] K. Benitz and H.F. Diermeier, *Proc. Soc. Exp. Biol. Med.* **115**, 930–935 (1964).
[11] N.H. Khan, P. Wera, E. Roets and J. Hoogmartens, *J. Liq. Chromatogr.* **13**, 1351–1374 (1990).

[12] *European Pharmacopoeia*, 2nd edn, Monograph 210. Maisonneuve Sainte-Ruffine, France (1993).
[13] *United States Pharmacopeia XXII*. United States Pharmacopoeial Convention, Rockville, MD, USA (1990).
[14] *British Pharmacopoeia*. Her Majesty's Stationery Office, London (1988).

[*Received for review 18 February 1994; revised manuscript received 20 July 1994*]