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QUANTITATIVE ANALYSIS OF DOXYCYCLINE AND RELATED SUBSTANCES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Isocratic high-performance liquid chromatography on Hamilton PRP-1 column (25 × 0.46 cm I.D.) at 60°C allows the separation of oxytetracycline, 4,6-epidoxycycline, 4-epidoxycycline, methacycline, 6-epidoxycycline and doxycycline. The mobile phase is tetrahydrofuran–0.2 *M* phosphate buffer (pH 8.0)–0.2 *M* tetrabutylammonium hydrogen sulphate solution (pH 8.0)–0.1 *M* sodium edetate solution (pH 8.0)–water (6:10:5:1:78). The flow-rate is 1.0 ml/min, 40-μg samples are injected and detection is at 254 nm. The column lifetime exceeds 6 months. Results for a number of bulk samples and specialities are reported.

INTRODUCTION

A number of papers on high-performance liquid chromatography (HPLC) of tetracyclines mention the determination of doxycycline (DOX) in biological samples[1–8]. The systems described are less suitable for the determination of related substances because the separation of the antibiotic from the background of biological origin is the main concern. Other papers describe systems for the separation of DOX from other tetracyclines[9–17]. Such systems can be useful for the identification of DOX, but are not necessarily suitable for purity control and assay. Practically all the HPLC systems described are based on reversed-phase chromatography; only a few are based on ion-exchange chromatography[1,6].

Some of the systems mentioned in the literature are applicable to the purity control of DOX. Nelis and De Leenheer[19] described a reversed-phase system with a mobile phase of pH 2.1, able to separate DOX from the impurities 4-epidoxycycline (4-EDOX), 6-epidoxycycline (6-EDOX) and methacycline (MTC). The impurities themselves were not separated from each other. Gstrein and Nachtmann[20] were able to separate DOX, 6-EDOX, MTC and oxytetracycline (OTC) on a reversed-phase

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system with a mobile phase of pH 8.0. The separation of 4-EDOX was not mentioned. Another drawback of this system is the relatively high pH of the mobile phase combined with a relatively high temperature (50°C), which is known to degrade the packing material. More recently, Hermansson and Andersson[21] described a similar system operating at room temperature with a mobile phase of pH 8.0, with which they were able to separate DOX, 6-EDOX and MTC. De Meijer, who used this system for the determination of 6-EDOX in a number of commercial DOX samples, reported a satisfactory stability for the packing material. Results for 4-EDOX or MTC were not mentioned[22].

This paper describes a method that separates OTC, 4,6-epidoxycycline (4,6-EDOX), 4-EDOX, 6-EDOX, MTC and DOX.Under the conditions described, the Hamilton PRP-1 packing material is stable for more than 6 months. Results for a number of bulk samples, tablets, capsules and suspensions are reported. The W.H.O. international reference preparation, the U.S. reference standard and the European Pharmacopoeia chemical reference substance (Ph. Eur. CRS) are compared.

EXPERIMENTAL

Samples

More than 50 samples were analysed. For a number of samples the manufacturer of the DOX was known, *e.g.* Ankerfarm, Lark and Carbo-Biochimica (Italy), Hovione (Portugal), Pfizer (U.S.A.) and Rachelle (U.S.A.). For other samples, which were obtained from wholesale dealers of bulk products and from manufacturers of specialities, the manufacturer of the DOX was not always known. A reference sample containing 4-EDOX was kindly donated by Pfizer.

Chemicals

Organic solvents from Janssen Chimica (Beerse, Belgium) were distilled before use. Quaternary ammonium salts were from the same manufacturer. Other reagents were of *pro analysi* quality (Merck, Darmstadt, F.R.G.). Water was distilled twice.

HPLC apparatus and operating conditions

The HPLC apparatus consisted of a reciprocating pump Model M45 (Waters Assoc., Milford, MA, U.S.A.), a 20-μl loop injector Model CV-6-UHPa-N60 (Valco, Houston, TX, U.S.A.), a 250 × 4.6 mm I.D. column, packed in the laboratory with 10-μm Hamilton PRP-1 (Hamilton, Reno, NV, U.S.A.), a 254-nm fixed-wavelength detector Model 440 (Waters Assoc.), and a recording integrator Model 3390 A (Hewlett-Packard, Avondale, PA, U.S.A.). The column was kept at 60°C by means of a glass water-jacket, connected to a circulating water-bath. A Pye Unicam Model LC3UV variable-wavelength detector (Pye Unicam, Cambridge, U.K.) was used at 350 nm in some preliminary experiments where the mobile phase contained acetone.

The mobile phase was prepared by mixing 750 ml of water, 60 ml of tetrahydrofuran, 100 ml of 0.2 *M* phosphate buffer (pH 8.0), 50 ml of 0.02 *M* tetrabutylammonium hydrogen sulphate (TBA) solution and 10 ml of 0.1 *M* sodium edetate (EDTA). During preparation of the latter two solutions the pH was brought to 8.0 by the addition of sodium hydroxide solution. The mixture was finally diluted to 1000 ml with water. The flow-rate was 1.0 ml/min, and the back-pressure was *ca.* 1500 p.s.i.

Sample preparation

Samples were prepared by weighing or pipetting accurately an amount corresponding to 40 mg of DOX base into a 25-ml volumetric flask.

For bulk samples of salts of DOX, the mobile phase was used as the solvent. DOX base samples were first dissolved in 1.5 ml of 0.1 *N* hydrochloric acid, 20 ml of mobile phase were added, then 1.5 ml of 0.1 *N* sodium hydroxide, and finally further mobile phase was added.

For tablets and capsules containing a salt of DOX, 20 ml of mobile phase were added and the mixture was treated in an ultrasonic bath at room temperature for 5 min. Further mobile phase was then added. For samples containing the base, the sample was first treated with 2.5 ml of 0.1 *N* hydrochloric acid in an ultrasonic bath at room temperature for 5 min, 20 ml of mobile phase were added and the mixture was sonicated for 1 min, 2.5 ml of 0.1 *N* sodium hydroxide were added, and finally further mobile phase was added. Part of the homogeneous mixture was centrifuged, and the supernatant was filtered through a membrane filter with 1.2-μm pores.

For the suspensions, 5.0 ml of 0.1 *N* hydrochloric acid, 5 ml of 0.1 *M* sodium edetate solution (pH 8.0), 5.0 ml of 0.1 *N* sodium hydroxide, and finally further mobile phase, were added.

Calibration curves and reproducibility

Calibration curves were obtained with chemical reference substances of the European Pharmacopoeia (y = peak area, x = amount injected in micrograms) DOX hyclate: $y = 0.12897x - 0.036$, $S_{y,x} = 0.088$, $r = 0.999$, range of x covered in the experiments: 30–50 μg. 6-EDOX hydrochloride: $y = 0.12x$, $r = 0.999$. MTC hydrochloride: $y = 0.16x$, $r = 0.998$. Range of x covered in 6-EDOX and MTC experiments: 0.02–0.8 μg.

A sample was analysed 22 times over a period of 8 days. The relative standard deviation (R.S.D.) for DOX was 0.6% and that for minor impurities such as 6-EDOX and MTC did not exceed 2%.

RESULTS AND DISCUSSION

DOX (Fig. 1) is probably the most stable product of the group of tetracyclines. Since the molecule carries a hydroxyl group at C-5 it is, like OTC, more stable towards C-4 epimerisation. This is probably due to hydrogen bonding of the hydroxyl with the C-4 dimethylamino group[23]. The absence of a hydroxyl at C-6 excludes the possibility of acid degradation to the corresponding anhydro derivative[24]. This means that, unlike TC and CTC, DOX must not be analysed for anhydro derivatives. On the other hand, since DOX is a semisynthetic product, it must be analysed for intermediates or secondary products. DOX can be prepared from OTC, MTC being an intermediate product. Therefore MTC, and to a lesser extent OTC, can be present as impurities. The C-6 epimer 6-EDOX can also be formed during the transformation of MTC into DOX[25]. Eventually 6-EDOX can epimerise into 4,6-EDOX. The method described enables the separation of all those substances.

The stationary phase used is a polystyrene–divinylbenzene (PSDVB) copolymer. This material is known for its good stability under extreme pH conditions (pH 1–13). PSDVB materials have been widely used in classical open-column chromato-

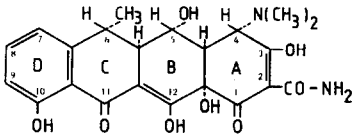

Fig. 1. Structure of doxycycline.

graphy, mainly as the well-known Amberlite XAD resins. HPLC separations with ground XAD resins have also been described, but more recently PSDVB materials with suitable cross-linking and particle size, designed for HPLC, have become available. Hitachi gels (Hitachi, Tokyo, Japan) have been used for the separation of alkylbenzenes, fatty acid alkanolamines, steroids, bases, polymyxin antibiotics and peptides[26–31]. Hamilton PRP-1, the packing material used in this work, was introduced more recently[32–34], and applications with chlorophenols, gibberellins and peptides have been described [35–38]. It seems that, despite their better stability at higher pH, PSDVB materials are not yet widely used in HPLC, which is probably due to their lower efficiency. It will be shown below that, for DOX at least, the lower efficiency is largely compensated for by a very good selectivity.

In accordance with the systems previously described[20,21] for silica-based reversed-phase materials, slightly alkaline (pH 8–9) mobile phases containing phosphate buffers and amines such as triethylamine, dimethyloctylamine, ethanolamine and tris(hydroxymethyl)aminomethane were used initially. It was observed that replacement of these amines by quaternary ammonium ions such as tetramethylammonium, tetraethylammonium or tetrabutylammonium (TBA) gave better separations. Solutions of TBA, neutralised with sodium hydroxide to the same pH as that of the phosphate buffer in the mobile phase, gave the most satisfactory results. Results obtained at pH 8.25 with different organic modifiers are shown in Fig. 2. As methanol gave poorer separations, it was excluded from further experiments. Fig. 3 shows the influence, at pH 8.0, of the concentrations of phosphate buffer and of TBA, with acetone as the organic modifier. A 0.2 *M* phosphate buffer concentration of 10% (v/v) and a 0.02 *M* TBA concentration of 5% (v/v) were finally chosen as adequate. Fig. 4 shows the influence of the pH with acetone, acetonitrile or tetrahydrofuran (THF) as the organic modifier. MTC and OTC were also included in these experiments. At pH 8.0 THF allowed complete separation of all the compounds. EDTA was added to the mobile phase later, because it was found to increase the chromatographic efficiency. The mobile phase finally used is described in the Experimental section. An internal standard was not considered useful because a fixed-loop injector was used and aqueous solutions were analysed. It was observed in our laboratory that an internal standard does not improve the results when solutions with a low coefficient of expansion are analysed. For other solutions the temperature of the injector, and thus the room temperature, play an important role. This influence can be compensated for by the use of an internal standard, provided the solutions are prepared at a well-defined temperature.

Fig. 5a shows a typical chromatogram of DOX. OTC and 4,6-EDOX are not present in detectable amounts ($\ll 0.1\%$). A very small amount ($< 0.1\%$) of 4-EDOX is present. MTC, 6-EDOX and an impurity of unknown structure, eluting after DOX, are present in most samples. This unknown peak will be designated as UNK. Fig. 5b

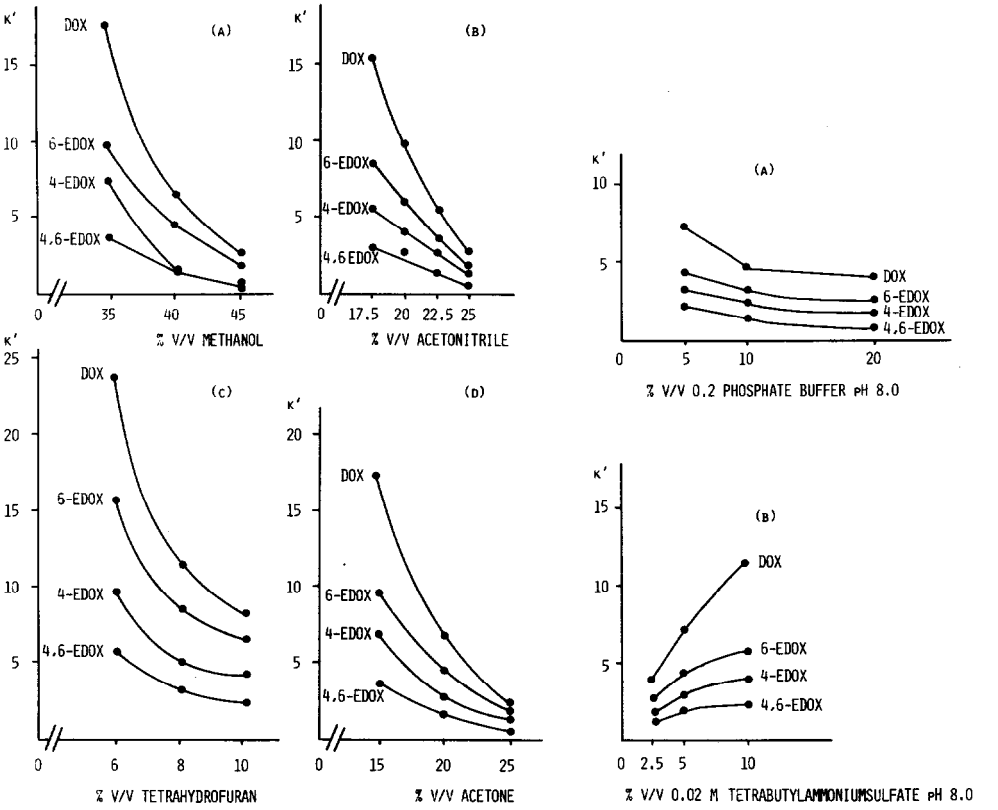

Fig. 2. Influence of the organic modifier on the separation of doxycycline and related substances: (A) methanol, (B) acetonitrile, (C) tetrahydrofuran, (D) acetone. Mobile phase: organic modifier–0.2 *M* phosphate buffer (pH 8.25)–0.02 *M* tetrabutylammonium sulphate solution (pH 8.25)–water [*x*:10:5:(85 − *x*)]. In experiments using acetone, detection was carried out at 350 nm. See Experimental section for other conditions.

Fig. 3. Influence of (A) buffer concentration and (B) quaternary ammonium ion concentration on the separation of doxycycline and related substances. Mobile phase: acetone–0.2 *M* phosphate buffer (pH 8.0)–0.02 *M* tetrabutylammonium sulphate solution (pH 8.0)–water, (A) 20:*x*:5:(75 − *x*), (B) 20:10:*x*:(75 − *x*). Detection at 350 nm. See Experimental section for other conditions.

shows a chromatogram of DOX polyphosphate. 4-EDOX is present in quite a large amount: 8% of the total doxycycline content. Epimerisation probably occurred during preparation of the polyphosphate derivative. Phosphate salts are known to increase the rate of epimerisation at C-4[39]. The same phenomenon was observed in our laboratory for capsules containing TC phosphate; 17-month-old samples were found to contain up to 14% of the C-4 epimer. C-4 epimers, which were used as reference products for chromatography, were prepared in solution by partial transformation of DOX and 6-EDOX in the presence of phosphate buffers. The products were not isolated. The 4-EDOX peak thus obtained corresponded to that of the reference sample containing 4-EDOX : DOX (2:1). It is clear that the PSDVB stationary phase shows a very good selectivity towards epimers of tetracyclines. The separation of

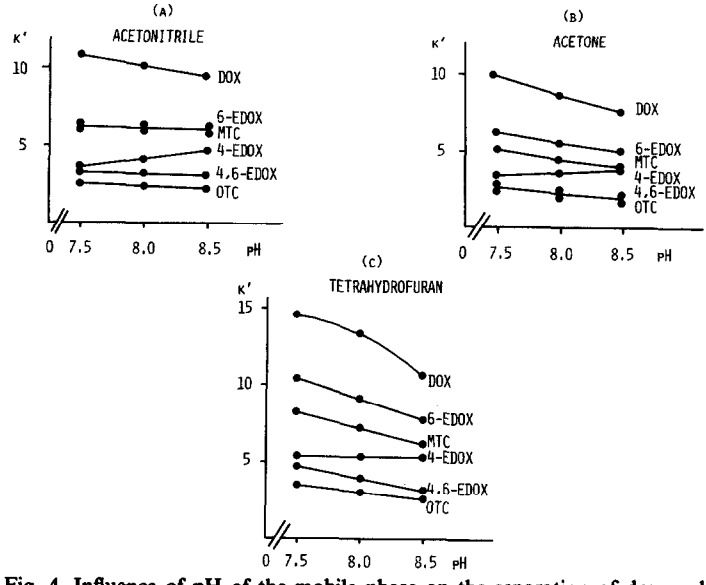

Fig. 4. Influence of pH of the mobile phase on the separation of doxycycline and related substances. Mobile phases contained 10% (v/v) 0.2 *M* phosphate buffer of the pH indicated and 5% (v/v) 0.02 *M* tetrabutylammonium sulphate solution, brought to the pH indicated with sodium hydroxide solution. Other components of the mobile phase were: (A) 20% (v/v) acetonitrile; (B) 17.5% (v/v) acetone; (C) 8% (v/v) tetrahydrofuran. Water was added to 100%. In experiments using acetone, detection was carried out at 350 nm. See Experimental section for other conditions.

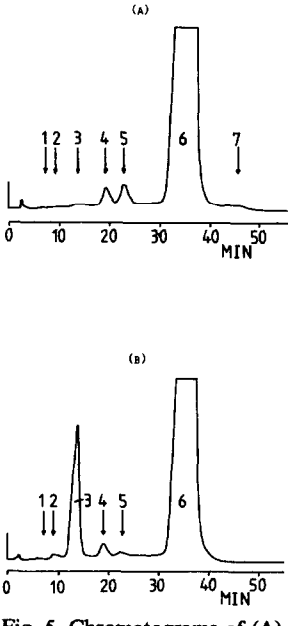

Fig. 5. Chromatograms of (A) a typical sample of doxycycline hyclate, (B) doxycycline polyphosphate. Column, Hamilton PRP-1, 250 × 4.6 mm I.D. at 60°C; mobile phase, tetrahydrofuran–0.2 *M* phosphate buffer (pH 8.0)–0.02 *M* tetrabutylammonium sulphate solution (pH 8.0)–0.1 *M* sodium edetate solution (pH 8.0)–water (6:10:5:1:78); flow-rate, 1.0 ml/min; detection wavelength, 254 nm. Peaks: 1 = oxytetracycline; 2 = 4,6-epidoxycycline; 3 = 4-epidoxycycline; 4 = methacycline; 5 = 6-epidoxycycline; 6 = doxycycline; 7 = unknown.

tetracycline epimers has always been a problem in HPLC on reversed-phase materials.

This HPLC method was used to compare the W.H.O. international reference preparation, the U.S. reference standard (F-1) and the Ph. Eur. chemical reference standard (Ph. Eur. CRS). A commercial sample, later used as a house standard, was examined at the same time. Samples were injected alternately over a period of 8 days. The secondary standard was analysed every day. For all the products examined the sum of the surfaces corresponding to MTC, 6-EDOX, DOX and UNK covered at least 99.9% of the total surface measured. The DOX · HCl content of the samples was obtained by comparison with the Ph. Eur. CRS. The composition of the Ph. Eur. CRS was calculated as follows. The manufacturer's values for water (1.88%) and ethanol (4.6%), corresponding to the total theoretical amount (6.48%) of solvent in a hyclate salt DOX · HCl · 0.5 H_2O · 0.5 C_2H_5OH, were checked in the Ph. Eur. laboratory and in our laboratory. Water was determined by Karl Fischer titration. The gas chromatographic determination of ethanol was that described in the Ph. Eur. for doxycycline hyclate[18]. Mean values were 2.32% (water) and 4.55% (ethanol). The MTC · HCl content (0.03%) and the 6-EDOX · HCl content (0.8%) were obtained by HPLC using calibration curves obtained with Ph. Eur. chemical reference substances. The remainder of the total mass [100% − (2.32% + 4.55% + 0.03% + 0.8%)] = 92.3% was accepted to correspond with the sum of the surfaces obtained for DOX and UNK. If UNK is calculated as DOX · HCl, a content of 0.2% is obtained, which means that the DOX · HCl content of the Ph. Eur. CRS is 92.1%. This value was used in the calculation of the content of the other standards and of all the other samples.

Results obtained for the standards are shown in Table I. The results show that

TABLE I

COMPOSITION OF DOXYCYCLINE STANDARDS

Values in percent (m/m); R.S.D. is given in parentheses; ND = not determined owing to limited amount of sample.

	Ph. Eur. CRS (872 I.U./mg)	*First International reference preparation (870 I.U./mg)*	*U.S. Reference standard (864 µg DOX/mg)*	*House standard*
Number of analyses	10	7	6	22
Number of solutions analysed	5	3	3	14
Number of days	5	3	3	8
MTC · HCl	0.03 (18.6)	0.2 (4.8)	0.2 (1.3)	0.7 (1.7)
6-EDOX · HCl	0.8 (5.1)	1.1 (5.1)	1.1 (0.6)	1.1 (1.3)
Unknown (UNK)	0.2	0.2	0.2	0.2
DOX · HCl	92.1 (0.6)	90.4 (0.5)	92.3 (0.3)	90.8 (0.6)
Subtotal	93.13	91.9	93.8	92.8
Ethanol	2.32	ND	ND	2.40
Water	4.55	ND	ND	4.85
Total	100.0			100.05
DOX	85.1	83.5	85.3	83.9

TABLE II

COMPOSITION OF BULK SAMPLES OF DOXYCYCLINE HYCLATE

Values in percent (m/m); ND = not determined owing to limited amount of sample.

Manufacturer	*Sample number*	*Number of analyses*	*Number of solutions analysed*	*Number of columns used*	*4-EDOX·HCl*	*MTC·HCl*	*6-EDOX·HCl*	*Unknown*	*DOX · HCl*		*Water**	*Ethanol*	*Total***
									Mean	*R.S.D. (%)*			
A	3	3	3	2	<0.1	1.1	0.9	0.2	90.8	0.4	1.8	4.6	99.4
	4	4	4	2	<0.1	0.2	0.9	0.2	91.4	0.5	2.0	5.0	99.4
	7	3	2	2	<0.1	0.3	1.1	<0.2	92.5	0.3	1.8	4.7	100.4
	10	3	3	2	<0.1	<0.1	1.1	0.2	93.1	0.5	2.0	4.8	101.2
B	11	3	3	2	<0.1	0.8	0.1	<0.2	93.3	0.7	1.9	4.4	100.5
	13	3	2	2	<0.1	0.6	0.4	≪0.2	93.9	0.7	2.0	4.2	101.1
C	15	4	3	2	<0.1	<0.1	1.2	<0.2	91.3	0.6	1.9	5.3	99.3
	17	4	3	2	<0.1	<0.1	0.4	0.3	92.7	0.7	2.1	4.5	100.0
	30	3	3	1	<0.1	0.2	0.3	0.4	92.3	0.5	2.2	4.6	100.0
D	19	3	2	2	≪0.1	≪0.1	0.5	0.4	92.6	0.2	2.0	ND	ND
	24	3	2	2	≪0.1	≪0.1	0.4	0.4	92.8	0.3	1.9	ND	ND
E	20	3	2	2	0.1	<0.1	0.3	<0.2	92.7	0.4	2.3	ND	ND
F	21	3	2	2	≪0.1	0.8	≪0.1	0.2	93.1	0.6	2.0	4.5	100.4
Unknown***	14	3	2	2	0.1	0.1	0.6	≪0.2	91.8	0.6	1.9	5.2	99.7
	16	3	2	2	<0.1	<0.1	1.5	0.2	90.8	0.4	2.0	5.3	99.8
	28	3	3	1	≪0.1	≪0.1	0.4	0.4	92.7	0.1	2.0	4.6	100.1
	29	6	5	1	≪0.1	≪0.1	1.4	<0.2	92.0	0.6	1.9	5.2	100.5

* Karl Fischer.

** Values given as < are not included.

*** Unknown because samples were not obtained from the manufacturer but from wholesale dealers.

the Ph. Eur. CRS and the U.S. reference standard contain comparable amounts of DOX, whereas the international standard contains somewhat less. The Ph. Eur. CRS contains the smallest amount of MTC and 6-EDOX. For the UNK content no R.S.D. is given because, in a number of experiments, the integrator did not count the small broad peak. The DOX base content is also mentioned.

The water content of the house standard was found to be 2.40% and the ethanol content 4.85%. When this solvent content is added to the subtotal of 92.8 mentioned in Table I a total of 100.05% is obtained. Owing to the limited amounts available, the water and ethanol contents of the other standards were not determined.

Table II shows results obtained with a number of hyclate salts. The number of samples taken is mentioned in the column "number of solutions analysed". Most of the samples were analysed on two different PRP-1 columns. In total three columns were used, and all three gave comparable separations. The R.S.D. for DOX · HCl is always less than 1%. OTC and 4,6-EDOX were never detected in commercial samples. Except for the phosphate salt shown in Fig. 5b, 4-EDOX is found to be a minor impurity. Since no pure reference material was available 4-EDOX was calculated as DOX · MTC, and 6-EDOX and the impurity of unknown structure (UNK) are present in variable amounts in all the samples. All the samples comply with the Ph. Eur. TLC test for related substances: OTC · HCl, 1%; MTC · HCl, 2%; 6-EDOX · HCl, 2%. It is seen that the 6-EDOX · HCl content is not always less than 0.5%, as was observed previously in a study covering more than 30 samples[22]. Some variation in composition is observed between samples from the same manufacturer. The composition pattern does not distinguish samples from different manufacturers. The water content is always close to the theoretical amount (1.88%) and within Ph. Eur. limits (1.4–2.8%). The ethanol content is also close to the theoretical value (4.6%) and well within Ph. Eur. limits (4.3–6.0%). In the samples from manufacturer B, *ca.* 0.5% of acetone was detected by the same gas chromatographic method. The total content is close to 100%. The mean of the total contents is 100.1%, with the R.S.D. 0.6%.

Table III shows results obtained for DOX · H_2O samples. The purity of the sample examined is comparable with that observed for the DOX salts. The water content is close to the theoretical value (3.89%).

Table IV gives the DOX content for a number of tablets, capsules and suspensions from different manufacturers. During sample preparation of the suspensions, DOX is first dissolved in hydrochloric acid, EDTA is added before the pH is brought to 8.0. This is necessary because the suspensions examined contain alkaline earth metals, which are known to form complexes with tetracycline[40]. The results for the capsules from manufacturer A fluctuate between *ca.* 93% and *ca.* 100%. This can be partly explained by the fact that sample 34 was manufactured in another plant, but samples 40 and 41, differing by as much as 6%, appeared to be from the same plant. The distinctly higher content of samples 37 and 39 is probably due to overdosing at 105%. Some fluctuation in the content of pharmaceutical preparations, although to a lesser extent, was also observed in a previous survey[22].

It can be concluded that the method described is useful for simultaneous identification, purity control and assay of bulk samples and different kinds of pharmaceutical preparation containing DOX. During preliminary experiments the same method, eventually slightly adapted, also proved to be useful for the analysis of other tetracyclines.

TABLE III

COMPOSITION OF BULK SAMPLES OF DOXYCYCLINE MONOHYDRATE

Values in percent (m/m).

Manufacturer	*Sample number*	*Number of analyses*	*Number of solutions analysed*	*Number of columns used*	*4-EDOX*	*MTC*	*6-EDOX*	*Unknown*	*DOX · H_2O*		*Water**
									Mean	*R.S.D. (%)*	
A	32	3	3	1	<0.1	1.0	0.5	<0.2	99.1	0.5	4.2
	33	2	2	1	<0.1	0.7	0.4	<0.2	100.0	0.2	4.1
C	31	2	2	1	≪0.1	≪0.1	0.4	<0.2	100.3	0.4	4.2
Unknown**	18	11	8	2	≪0.1	≪0.1	0.3	0.3	100.4	0.9	3.9

* Karl Fischer.

** Unknown because the sample was not obtained from a manufacturer but from a wholesale dealer.

TABLE IV

DOXYCYCLINE CONTENT OF SPECIALITIES AS A PERCENTAGE (m/m) OF LABEL CLAIM

Manufacturer	*Sample number*	*DOX derivative used*	*Form*	*Number of analyses*	*Number of solutions analysed*	*Mean content*	*R.S.D. (%)*
A	36	Monohydrate	Tablets	2	2	93.6	0.4
	34	Hyclate	Capsules	5	3	92.4	1.7
	40	Hyclate	Capsules	2	2	99.5	0.2
	41	Hyclate	Capsules	2	2	93.5	0.1
	44	Hyclate	Suspension	2	2	98.4	0.3
	45	Hyclate	Suspension	2	2	95.8	0.4
G	35	Monohydrate	Tablets	4	3	99.9	0.2
	37	Hyclate	Capsules	2	2	105.8	0.1
H	38	Hyclate	Capsules	2	2	103.4	0.1
I	39	Hyclate	Capsules	2	2	107.0	0.3
J	42	Hyclate	Capsules	2	2	102.4	0.6
K	43	Hyclate	Capsules	2	2	102.0	0.3
L	48	Hyclate	Capsules	2	2	101.0	0.3

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REFERENCES

1 A. P. De Leenheer and H. J. C. F. Nelis, *J. Pharm. Sci.*, 68 (1979) 999.
2 R. Böcker and C. J. Estler, *Arzneim.-Forsch.*, 29 (1979) 1690.
3 R. Böcker, *J. Chromatogr.*, 187 (1980) 439.
4 S. Eksborg, H. Ehrsson and U. Lönroth, *J. Chromatogr.*, 185 (1979) 583.
5 H. Jaeger, D. Russmann, F. Follath, M. Wenk, J. Pijck, Th. Hallynck and L. Dettli, *Arzneim.-Forsch.*, 30 (1980) 1932.
6 H. J. C. F. Nelis and A. P. De Leenheer, *J. Pharm. Sci.*, 70 (1981) 226.
7 R. Böcker and C. J. Estler, *Arzneim.-Forsch.*, 31 (1981) 2116.
8 R. Böcker, *J. Chromatogr.*, 274 (1983) 255.
9 K. M. Lotscher, B. Brander and H. Kern, *Varian Intruments Applications*, 9, No. 1 (1975) 12.
10 K. Tsuji, J. H. Robertson and W. F. Beyer, *Anal. Chem.*, 46 (1974) 539.
11 E. R. White, M. A. Carroll, J. E. Zarembo and A. J. Bender, *J. Antibiot.*, 28 (1975) 205.
12 A. P. De Leenheer and H. J. C. F. Nelis, *J. Chromatogr.*, 140 (1977) 293.
13 E. R. White, M. A. Carroll and J. E. Zarembo, *J. Antibiot.*, 30 (1977) 811.
14 G. D. Mack and R. B. Ashworth, *J. Chromatogr. Sci.*, 16 (1978) 93.
15 S. Eksborg, *J. Chromatogr.*, 208 (1981) 78.
16 S. Eksborg and B. Ekqvist, *J. Chromatogr.*, 209 (1981) 161.
17 A. Aszalos, C. Haneke, M. J. Hayden and J. Crawford, *Chromatographia*, 15 (1982) 367.
18 *European Pharmacopoeia*, 2nd Edition, Maisonneuve, Sainte-Ruffine, France.
19 H. J. C. F. Nelis and A. P. De Leenheer, *J. Chromatogr.*, 195 (1980) 35.
20 K. Gstrein and F. Nachtmann, *Acta Pharm. Technol.*, 26 (1980) 247.
21 J. Hermansson and M. Andersson, *J. Pharm. Sci.*, 71 (1982) 222.
22 P. J. J. De Meijer, *Pharm. Weekbl.*, 117 (1982) 1018.
23 D. A. Hussar, P. J. Nierbergall, E. T. Sugita and J. T. Doluisio, *J. Pharm. Pharmacol.*, 20 (1968) 539.
24 J. R. D. McCormick, E. R. Jensen, P. A. Miller and A. P. Doerschuk, *J. Amer. Chem. Soc.*, 82 (1960) 3381.

25 C. R. Stephens, J. J. Beereboom, H. H. Rennhard, P. N. Gordon, Kotaro Murai, R. K. Blackwood and M. Schach von Wittenau, *J. Amer. Chem. Soc.*, 85 (1963) 2643.
26 A. Nakae and G. Muto, *J. Chromatogr.*, 120 (1976) 47.
27 A. Nakae and K. Kunihiro, *J. Chromatogr.*, 156 (1978) 167.
28 R. Horikawa, T. Tanimura and Z. Tamura, *J. Chromatogr.*, 168 (1979) 526.
29 K. Aramaki, T. Hanai and H. F. Walton, *Anal. Chem.*, 52 (1980) 1963.
30 Y. Kimura, H. Kitamura, T. Araki, K. Noguchi, M. Baba and M. Hori, *J. Chromatogr.*, 206 (1981) 563.
31 T. Isobe, Y. Kurosu and T. Okuyama, *Protides Biol. Fluids*, 30 (1982) 727.
32 D. P. Lee and J. H. Kindsvater, *Anal. Chem.*, 52 (1980) 2425.
33 D. P. Lee, *J. Chromatogr. Sci.*, 20 (1982) 203.
34 Z. Iskandarani and D. J. Pietrzyk, *Anal. Chem.*, 54 (1982) 1065.
35 C. E. Werkhoven-Goewie, W. M. Boon, A. J. J. Praat, R. W. Frei, U. A. Th. Brinkman and C. J. Little, *Chromatographia*, 16 (1982) 53.
36 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
37 R. I. Greyson and A. M. Patch, *J. Chromatogr.*, 242 (1982) 349.
38 T. Sasagawa, L. H. Ericsson, D. C. Teller, K. Titani and K. A. Walsh, *J. Chromatogr.*, 307 (1984) 29.
39 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. Amer. Chem. Soc.*, 79 (157) 2849.
40 A. Albert and C. N. Rees, *Nature (London)*, 177 (1956) 433.