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A quantitative chromatographic method for the determination of purity of oxytetracycline

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A chromatographic method for the determination of oxytetracycline is described, employing a hexane–ethyl acetate–pH 3·3 buffer partition system supported on Celite. Chromatographic separation is followed by measuring the absorbance of the eluate at 263 nm. The system enables the determination of oxytetracycline to be made in the presence of anhydro-oxytetracycline, chlortetracycline, α- and β-apo-oxytetracycline, and epi-oxytetracycline, the last three being the most likely contaminants of crude material.

The method currently employed for the assay of oxytetracycline (B.P. 1968) uses standard microbiological procedures and *Bacillus pumilus* as test organism. An accuracy of ± 2% is obtained only if multiple determinations are made on three successive occasions making the method expensive in its use of laboratory space and personnel. The method is also non-specific in that any impurities present which show activity against the test organism, will be estimated as oxytetracycline. Previous attempts have been made to assay oxytetracycline by methods based on the estimation of a specific functional group or physical properties of the oxytetracycline molecule, viz. by ultraviolet absorption spectroscopy, by measurement of the colour produced with ferric chloride (Monastero, Means & others, 1951), by means of fluorimetric analysis (Serembe, 1951) or polarography (Doshocil, 1954). All suffer serious interference from the structurally similar impurities likely to be present and the correlation between the results obtained by these procedures and those of the official microbiological assay, has been poor.

The physical properties of oxytetracycline and some of the likely contaminants are listed in Table 1. In view of their close similarity, preliminary separation of the oxytetracycline from other likely impurities is obviously essential in any analysis specific to this substance. Previous experience with partition chromatography on Celite suggested a possible solution to the problem.

EXPERIMENTAL

Reagents

Prepared Celite. Stir Celite 545 (500 g) intermittently (12 h) with concentrated hydrochloric acid (2 litres). Decant the hydrochloric acid and suspend the residue in water (1 litre). Filter through a Buchner funnel, wash the residue with water until free from acid, wash with methanol (500 ml) then methanol–ethyl acetate (1 : 1) (1 litre). Dry the residue in an oven at 100°. Store in well-stoppered jars.

pH 3·30 *Buffer.* Dissolve an accurately weighed quantity of Analar potassium hydroxide pellets (120 g) in water (600 ml). Dilute an accurately measured volume of Analar syrupy phosphoric acid (120 ml) to about 600 ml with water. Mix the two

solutions, allow to cool and adjust the volume to 2 litres with water. Adjust the pH of this solution accurately to 3·30 by the addition of more potassium hydroxide or syrupy phosphoric acid as required.

Solvent system. Shake together ethyl acetate Analar (675 ml), n-hexane (325 ml) and pH 3·3 buffer (100 ml) and allow to separate. The upper layer is the eluent phase, the lower layer the stationary phase.

Table 1. *Physical properties of oxytetracycline and some likely impurities*

Compound	M.p. °C	Ultraviolet absorption Max nm	Log ε	Infrared absorption	Specific rotation $[\alpha]_D^{20}$
Oxytetracycline anhydrous	184–185	222 270 364 Acid	4·15 4·30 4·16 Ethanol	KBr disc 1675 cm^{-1}	−197° (0·01 HCl)
Anhydro-oxytetracycline ..	180–190	271 425 Acid	4·56 3·80 Ethanol	KBr disc 1670 cm^{-1}	+52° (1 : 1 Methanol Dioxan)
α-Apo-oxytetracycline ..	190–200	250 377 Acid	4·77 3·87 Ethanol	KBr disc 1720 cm^{-1}	−45° (D.M.F.)
β-Apo-oxytetracycline ..	195–205	248 375 Acid	4·78 4·0 Ethanol	KBr disc 1740 cm^{-1}	−28° Ethanol
Chlortetracycline	168–169	234 269 376 Acid	4·22 4·25 4·10 Ethanol	KBr disc 1670 cm^{-1}	−245° (0·03N HCl)
Epioxytetracycline	163–164	215 253 275 355 0·1N sulphuric acid	4·09 4·15 4·08 4·08	KBr disc 1680 cm^{-1}	−253° (0·03N HCl)

Preparation of sample and standard solutions. Dissolve about 50 mg accurately weighed in 0·1N hydrochloric acid (10 ml), adjust the pH to approximately 7 with 0·1N sodium hydroxide solution (approx. 11 ml) and immediately dilute to 50 ml in a volumetric flask with stationary phase. Prepare a standard solution of pure anhydrous oxytetracycline base in an identical manner.

Method. Mix prepared Celite (25 g) with stationary phase (12·5 ml) and pack into a chromatographic column (70 cm × 2·2 cm internal diameter fitted with a sinter plate) in portions of about 3 g, tamping down firmly between each addition. To a further 2 g of prepared Celite add sample solution (1·0 ml), mix thoroughly and transfer the mix quantitatively to the top of the stationary phase in the column. Carefully add eluent phase until the stationary phase is covered to a depth of about 50 cm and adjust the flow of eluate from the column to about 10 ml/75 s. Collect 50 successive 10 ml fractions of eluate in 6 × 1 inch stoppered test tubes. Measure the absorbance at 263 nm (1 cm path length) of each fraction against eluent phase in the reference cell. Repeat the chromatogram using 1·0 ml of standard oxytetracycline solution.

The per cent oxytetracycline in the sample $= \frac{\text{Ea. Wg. } 100}{\text{Eg. Wa}}$ where Ea and Eg

are the sums of the absorbance values under the sample and standard peaks respectively. Wa = weight of sample (mg), Wg = weight of standard oxytetracycline anhydrous base (mg).

RESULTS AND DISCUSSION

Samples of anhydro-oxytetracycline, α- and β-apo-oxytetracycline, chlortetracycline, and epi-oxytetracycline were assayed by the above procedure, and the relations between absorbance at 263 nm and volume of eluate for two of these are illustrated in Fig. 1. For the other three compounds, anhydro-oxytetracycline proved

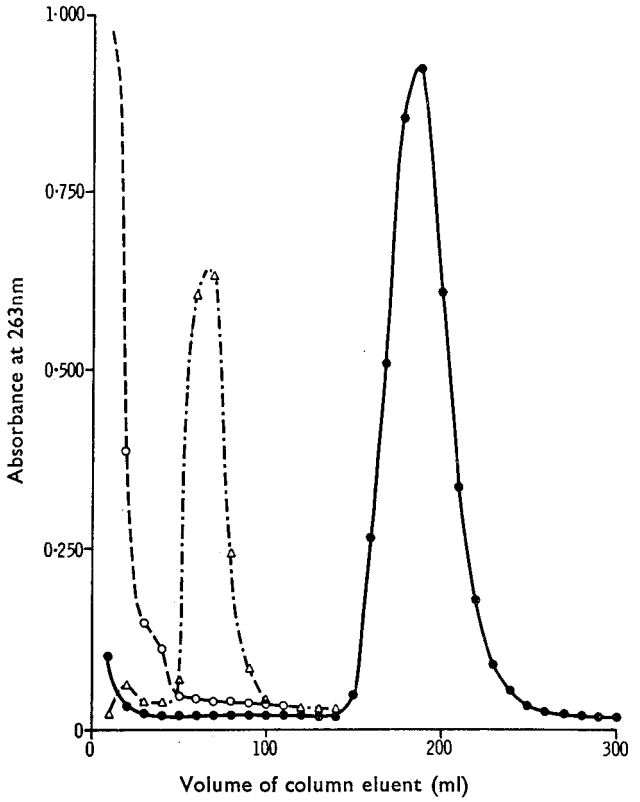

FIG. 1. Graph relating absorbance and retention volume for oxytetracycline and possible impurities. ○ — — — — ○, β-apo-oxytetracycline; △ — · — · — △, chlortetracycline and ●———●, oxytetracycline.

to be so labile in the presence of dilute acid (used in the first stage of the sample preparation) that it was converted quantitatively to a mixture of α- and β-apo-oxytetracycline. α-Apo-oxytetracycline and epi-oxytetracycline retained on the column were not eluted after 600 ml. Thus none of the above compounds interfere with the assay of oxytetracycline by the method described.

The proposed method has been applied to a series of samples of both pharmaceutical and crude grades of oxytetracycline and to material isolated from mother liquors at various stages of the purification process. The results compared with those using the microbiological assay (B.P. 1968) are shown in Table 2 and illustrate

Table 2. *Comparison of results obtained using the proposed method with those of the microbiological assay*

Sample	Potency calculated to Oxytetracycline base: Microbiological (±2%)	Chromatographic (±1%)*
Laboratory prepared samples	974	980
	972	980
	981	983
	983	990
	988	990
	Potency calculated to oxytetracycline dihydrate	
Crude Base	847	833
	820	818
	842	837
Pharmaceutical Grade	899	898
	869	875
	916	904
	912	892
	Potency calculated to oxytetracycline hydrochloride	
Pharmaceutical Grade	851	854
	850	832
	889	863
	903	895
	Potency calculated as calcium salt	
Pharmaceutical Grade	873	872
	849	849
	824	817
	International standards	
	Declared microbiological potency	Chromatographic
1st Int. Standard	900	890
		903
2nd Int. Standard	880	880
		878
		876
		880

* Based on 10 replicate determinations.

the value of the method for both control and pharmaceutical grade oxytetracycline and process development studies. Much information is gained about the mixture and quantity of any impurity present and although the method described is not recommended for the accurate determination of trace impurities in the pharmaceutical grade, simple adaptation of the technique makes this possible.

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