Short Communication

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Non-Aqueous Titrimetric Determination of Oxytetracycline Hydrochloride

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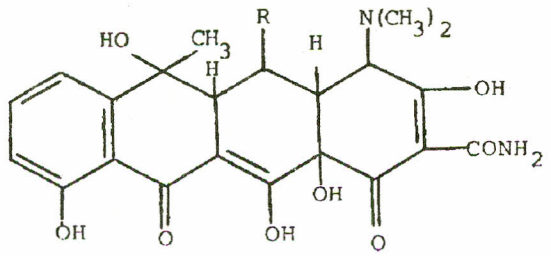
Oxytetracycline (R=OH) and tetracycline (R=H) are broad-spectrum antibiotic [1,2]. Oxytetracycline including tetracycline can be determined by microbiological [3], chromatographic [4-6], spectrophotometric [7-11], flourimetric [12–15], titrimetric [16-17], flow-injection [18] and electro-chemical methods [19-20].



where R=H or R=OH

Microbiological assay is sensitive, but requires a long period of incubation and lacks precision and specificity. Spectrophotometric methods are rather insensitive due to interference from other materials [14]. Other methods involve elaborate instrumentation or have low sampling frequency.

This paper describes a non-aqueous titrimetric method for the determination of oxytetracycline hydrochloride based on the libration of the drug as a free base followed by its extraction in chloroform and titrating the base with perchloric acid in dioxane or acetic acid.

Pure oxytetracycline hydrochloride or the solid dosage form of the drug was weighed as such or by emptying the capsule to contain 150 mg or suitable quantity of the oxytetracycline hydrochloride. To this was then added 10 ml of distilled water, transferred to a separating funnel completely with the addition of a few millilitres of water and was saturated with about 2gm of sodium chloride. One ml of 20% sodium hydroxide solution was added to render the system basic completely. The base was extracted with three times 15 ml of chloro form. The combined chloroform extracts were washed with 10 ml water and the aqueous washings were re-extracted with further 10 ml of chloroform. The extract was combined with the original extract. The chloroform extract was made free from water droplets by filtering through a plug of cotton wool supporting a layer of anhydrous sodium sulphate. The dry chloroform extract was then titrated with 0.1M perchloric acid in dioxane. The end point was obtained either potentiometrically noting the inflexion point or following the titration to blue end point in presence of one drop of crystal violet indicator.

Each ml of 0.1 M perchloric acid is equivalent to 49.69 mg oxytetracycline hydrochloride.

The following relationship may also be used to determine the amount of the drug.

$$gm/litre = E \times N$$

where E = Equivalent weight of the drug.

N = Normality of the drug present in solution form.

Titration of the extracted oxytetracycline hydrochloride with perchloric acid was carried out using glass calomel electrode system. It showed a characteristic curve as shown in Fig. 1. The electrode potential (mV) was found to rise with increasing amount of perchloric acid till a plateau was obtained. The millilitres of titrant corresponding to the mid-wave potential were taken as readings for equivalent point. A simi-

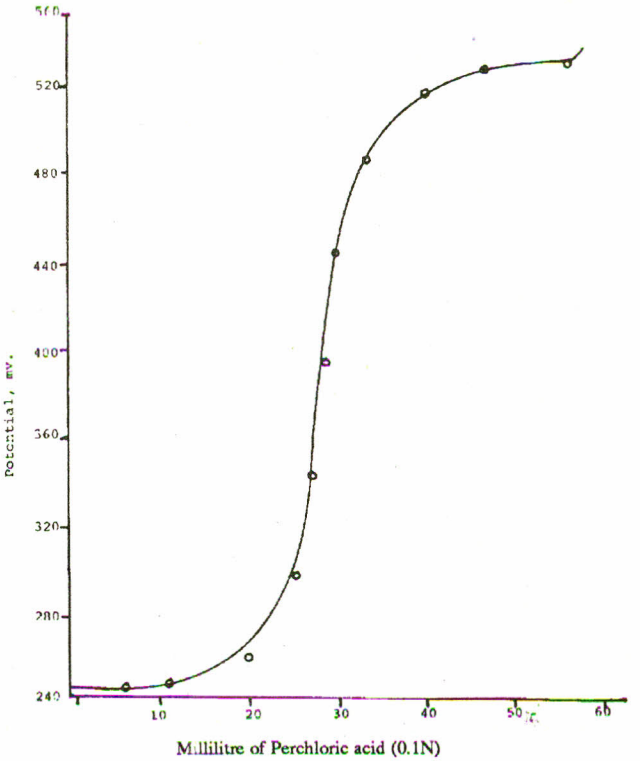


Fig. 1. Measurement of potentials (mV) as a function of ml of titrant reacting with oxytetracycline hydrochloride.

lar curve for oxytetracycline hydrochloride was obtained by following the procedure [21] described for the determination of methacoline chloride, based on its reaction with mercuric acetate followed by titration with perchloric acid.

The suitability of the method was ascertained by the determination of oxytetracycline in samples from pharmaceutical products, e.g., capsules. Average recoveries of 5 determinations for each form of the sample are given in Table 1. A quantity varying from 50-200 mg of oxytetracycline hydrochloride was determined with recovery of 97-98%. The proposed method was found comparable to the mercuric acetate method [21]. Table 2 lists the results obtained by the 2 methods. Reproducibility, or precision is expressed as RSD (Relative Standard Deviation) from several forms each of 5 analysis.

The above mentioned procedure was applied to the determination of other related compound i.e. salt (hydrochloride) of the base form. The reaction offered promising results for Vitamin B_1 and B_6 estiminations as illustrated in Table 3. Here the vitamins have been determined quantitatively with a % recovery around 99.

Null hypothesis testing of the results obtained by the two methods. One way in which a new analytical method may be tested is to compare the mean result, $\bar{x}_1$, obtained using the method with the mean, $\bar{x}_2$, obtained using a second (standard method). The null hypothesis is that there is no systematic difference between the two methods. If this hypothesis is true then $\bar{x}_1 - \bar{x}_2$ should not differ significantly from zero. Assuming that the two methods do not differ in precision, the standard deviation of the two samples, S_1 and S_2, can be pooled to give an overall estimate of standard deviation:

$$S^2 = (n_1-1)\, S_1^2 + (n_2-1)\, S_2^2 \quad \sqrt{(n_1-1 + n_2-1)}$$

and then t is given by:

$$t = (\bar{x}_1 - \bar{x}_2) / S \sqrt{(1/n_1 + 1/n_2)}$$

where t has $(n_1-1 + n_2-1)$ degrees of freedom.

TABLE 1. DETERMINATION OF OXYTETRACYCLINE HYDROCHLORIDE PRESENT IN PHARMACEUTICAL PRODUCTS (CAPSULE).

Product	Sample size (mg)	Average* found (mg)	Recovery (%)	Standard deviation (%)
Oxytetracycline	50	49.0	98	0.50
Hydrochloride	100	98.0	98	0.45
(Pfizer)	150	144.1	96	0.30
	200	194.0	97	0.62

*Average 5 determinations.

TABLE 2. QUANTITATIVE COMPARATIVE ANALYSIS OF THE OXYTETRACYCLINE HYDROCHLORIDE; THE PROPOSED METHOD VERSUS THE MERCURIC ACETATE METHOD.

Product	Sample size (mg)	Recovery* and relative standard deviation: Proposed method	Mercuric acelate method	$t = \frac{(\bar{x}_1 - \bar{x}_2)}{[S\sqrt{(1/n_1 + 1/n_2}]}$
Oxytetracycline chloride Capsule (Pfizer)	50	49.14 ± 0.1719	49.05 ± 0.2943	0.592
	100	99.10 ± 0.2774	98.90 ± 0.2769	1.141
	150	146.70 ± 0.9535	145.65 ± 0.7282	1.900
	200	197.40 ± 1.0857	196.40 ± 0.8830	1.602

* Average of 5 determinations.

TABLE 3. APPLICATION OF THE PROPOSED METHOD TO THE DETERMINATION OF VITAMIN B_1 AND B_6.

Vitamins	Amount taken (mg)	Amount found (mg)	Recovery (%)	Standard deviation (%)
B_1	100	97.9	97.9	0.65
	150	147.0	98.0	0.30
	200	199.1	99.5	0.39
B_6	100	98.68	98.7	0.71
	150	148.03	98.7	0.20
	200	196.00	98.0	0.35

*Average of 5 determinations.

The results obtained in Table 2 are based on the above approaches. The null hypothesis is that the methods give the same result. For the two methods, x_1, x_2, S_1 and S_2 are given in the Table. The values of 't' have accordingly been calculated and presented in the Table 2.

The critical value of [t] for 4 degrees of freedom is 2.7764 (P=0.05) so there is no evidence of a systematic difference between the methods for the four samples sizes studied here.

Key words: Non-aqueous titration, Oxytetracycline hydrochloride, Extraction.

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