ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 357–359

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

Field method for the micro-quantitative determination of tetracycline in human blood serum

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Received 5 November 1997

Keywords: Determination; Human blood serum; Tetracycline; Titrimetric method

1. Introduction

Tetracycline is an important antibiotic and its quantitative estimation has been of great interest to researchers for many decades. Among the various analytical methods available for the analysis of tetracycline in human serum are liquid chromatography [1,2], high-performance liquid chromatography [3,4], spectrophotometry [5,6], fluorimetry [7,8], stopped-flow mixing technique [9,10], luminescence spectrometry [11] and differential-pulse polarography [12]. Moreover, ion exchange resin beads have been used in colour reactions by Qureshi and co-workers [13–17]. Nevertheless, titrimetric methods of analysis are still very widely used owing to their simplicity and wide applicability [18–20]. Thus, titrimetric analyses have been carried out in our laboratories and recently reported in the literature [21,22].

In the present communication, we describe a sensitive and accurate titrimetric method for the micro-quantitative determination of tetracycline hydrochloride (TCHC) in human blood serum using Dowex 1X8 resin beads as detection medium.

2. Experimental

2.1. Reagents

TCHC (Synbiotics, India), sodium hydroxide (Qualigens, India), *m*-dinitrobenzene (*m*-DNB) (Fluka, guaranteed reagent), Dowex 1X8 (BDH, UK) and dimethyl sulphoxide (DMSO) (E. Merck India Ltd.) were used.

A stock solution of TCHC was prepared by dissolving 2.0 mg in 1.00 ml serum; 1.0% *m*-DNB was prepared in distilled ethanol.

2.2. Procedure

From the TCHC stock solution 0.50 ml was pipetted out and made up to the mark with distilled water in a 10 ml measuring flask. To

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PII S0731-7085(97)00249-5

aliquots containing 20–100 μg TCHC in a 50 ml beaker, 1 ml *m*-DNB, 2 ml DMSO and a small amount of Dowex 1X8 resin beads were added. The solutions were titrated using a microburette with 0.01 mol l^{-1} NaOH. A deep brown colour observed on the resin beads signifies the end-point.

2.3. Determination of tetracycline hydrochloride in clinical samples

Blood serum (1 ml) was drawn from five volunteers who received 250 mg capsules of TCHC every 6 h for 4 days, and diluted up to the mark with distilled water in a 10 ml volumetric flask. A 1 ml aliquot was then pipetted out from the flask and transferred into a 50 ml beaker. The recommended procedure was then applied.

3. Results

The calibration curve was constructed by taking solutions of various amounts of TCHC. Each solution was titrated with NaOH as described above and the end-point recorded. A straight line was obtained when the volume of NaOH was plotted against amount (μg) of TCHC. The calibration curve is given in Fig. 1. The equation

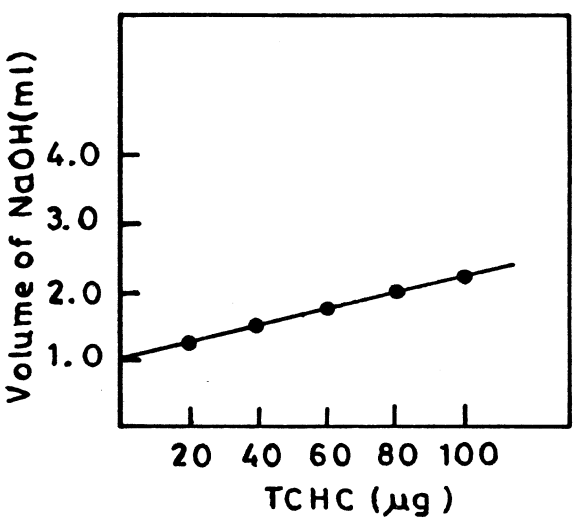

Fig. 1. Calibration curve for TCHC.

Table 1
Results obtained for the determination of tetracycline hydrochloride (TCHC) in clinical samples by the proposed method

Volunteer	Amount of TCHC (μg ml^{-1}) found in serum
A	2.27
B	2.43
C	2.19
D	2.35
E	2.11

followed is: Amount of TCHC (μg) = 81.03 × volume of NaOH (ml) − 81.80

The unknown amount of TCHC can be computed either from Fig. 1 or directly from the equation. The method was applied to the determination of TCHC in serum samples from ten healthy donors. The method was also successfully applied to the determination of TCHC in five clinical samples (Table 1). The literature reveals that the amount of tetracycline in serum drawn from patients who received 250 mg capsules every 6 h for 4 or more days was approximately 2–2.5 μg ml^{-1} [23,24]. Therefore, the results obtained in Table 1 are in agreement with the reported results.

4. Discussion

As already mentioned, chiefly due to the importance of TCHC, it was thought in the first instance to develop a specific titrimetric method for its determination in human serum, which can be used in routine analysis and provides a simpler and less time-consuming method than the existing ones. Therefore, a complementary method was devised to fulfil this purpose.

Spectroscopic observations have shown that tetracycline forms a charge-transfer complex with *m*-DNB [25]. On addition of NaOH, a charge-transfer complex between TCHC and *m*-DNB is formed first. When a slight excess of NaOH is added, *m*-DNB, like other polynitro aromatics, is expected to form an anionic sigma complex with bases [21,26]. This seems all the more likely because DMSO stabilizes the colour. The deep brown-coloured anionic sigma complex is ad-

sorbed onto the resin beads, indicating the end-point.

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