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

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ABSTRACT

This method described the determination of tetracycline in blood serum and it is based on the formation of an Eu(III)-tetracycline complex. Another spectrophotometric method[2] utilizing the reaction of tetracycline with p-N, N-dimethylphenylenediamine and chloramines T was described. A sensitive liquid chromatographic method (10) has been adopted for the simultaneous determination of tetracycline, oxytetracycline and minocycline in serum. Tetracyclines were separated from other serum components by RPLC with buffered MeOH mobile phase. UV absorbance of the column effluent was monitered at 267 nm.

Key words: Determination, tetracycline, urine and blood.

INTRODUCTION

Tetracycline is an important and its quantitative estimation has been of a great interest to researchers for many decades.

Among the various analytical methods available for the analysis of tetracycline in biological fluids, such as urine and serum, are spectrophotometry[1]. This method described the determination of tetracycline in blood serum and it is based on the formation of an Eu(III)-tetracycline complex. Another spectrophotometric method[2] utilizing the reaction of tetracycline with p-N, N-dimethylphenylenediamine and chloramines T was described. The tetracycline was then extracted by BUOH, and the absorbance measurement carried out at 640 nm. The method was applied for the estimation of tetracycline in blood and urine with a relative standard deviation ranging between 1.63-2.11 and recovery 98.0 – 99.3%. Essien *et al.* 3 performed a comparison between pulse polarographic and UV spectrophotometric methods for the analysis of tetracyclines in urine. The polarographic one combining d. c. and differential pulse polarography was more suitable than spectrophotometry at 353 and 365 nm for determining tetracycline and oxytetracycline, respectively. The ion-pair extraction of tetracyclines from body fluids using dyes as counter ions was also described[4]. Tetracyclines form ion-pair complex with dyes that can be extracted in $CHCl_3$ and re-extracted in HCl and finally quantified by spectrophotometry. 98% of tetracycline from the body fluids containing 10µg/ml of tetracycline can be extracted. High-performance liquid chromatography has also been used for the determination of tetracycline in biological fluids. Reeuwijk and Tjaden[5] have explored the possibilities of using two non-ionogeni resins (α AD-2 and PRP-1) in the chromatograph of tetracyclines and their degradation products in biological samples. Another HPLC procedure which allowed the rapid determination of tetracycline in blood was also described[6]. The detection limit was 10-500 ng/ml. Another HPLC method was also reported[7]. A rapid and accurate determination of tetracycline in human serum by reversed-phase HPLC with fluorescence detection was developed[8], based on protein precipitation in serum. The detection limits of this method were 10-35 ng/ml for three different tetracyclines. Tetracycline was also determined in

human urine by HPLC with PLRP-S column and guard column, a mobile phase of 7.5 mM H_3PO_4-MeCN-MeOH (20:33), and detection at 355 nm [9].

A sensitive liquid chromatographic method[10] has been adopted for the simultaneous determination of tetracycline, oxytetracycline and minocycline in serum. Tetracyclines were separated from other serum components by RPLC with buffered MeOH mobile phase. UV absorbance of the column effluent was monitered at 267 nm. Concentrations as low as 0.2 µg/m. of tetracyclines in serum were quantitable. Wenzel and co-workers [11] have discussed a liquid chromatographic and flow injection analysis for the determination of tetracycline in uring and serum using sensitized europium (III) luminescence detection. The method was highly selective for tetracycline since few compounds are capable for transferring energy to Eu(III). Novaka[12-13] has presented a thin layer chromatographic method for the identification of tetracycline in urine. Samples were extracted with Et acetate (with or without acidic hydrolysis) and the extractions were separated on Silufol plates using a mobile phase of Chloroform-methanol-0.1 M Na_2EDTA (55:30:5). The TLC spots were visualized by UV light at 254 nm or by spraying with aqueous solution of Fast Blue B. A differential pulse polarographic method for the determination of oxytetracycline in human urine and serum in acid media was also proposed[14]. The detection limit was 5.5×10^{-6} mol 1^{-1}. Adsorptive stripping voltammetric method[15] was also developed for the Quantative determination of tetracyclines in urine based on controlled adsorptive preconcentration of the antibiotic on the hanging mercury drop electrode (HMDE), followed by tracing the volatammogram in a cathodic potential scan. The modes used were d. c. stripping voltammetry (DCSV) and differential pulse striping voltammetry (DPSV). Fluorimetric methods have been extensively used for the determination of tetracycline in biological fluids and recently appeared in the literature[16-23]. Moreover, titrimetric methods of analysis and the use of ion exchange resin beads in color reactions are still very widely used owing to their simplicity and wide applicability[24-32].

In this chapter, we describe a sensitive and accurate titrimetric method for the micro-quantitative determination of tetracycline hydrochloride using Dowex 1 x 8 resin beads as detection. The methods has been successfully applied to human urine and serum samples.

EXPERIMENTAL

Reagents

Tetracycline hydrochloride (Synbiotics, India) 1, 3, 5-Trinitrobenzene (Fluka, guaranteed reagent), Sodium Hydroxide (Qualigens, India), Dowex 1 x 8 resin beads (BDH, England) and dimethyl sulfoxide (Emerk India Ltd.) were used.

Solutions

A stock solution of tetracycline hydrochloride was prepared by dissolving 2.0mg in1.00 ml urine. Another stock solution of tetracycline hydrochloride was prepared by dissolving 2.0mg in 1.00 ml serum 0.01 mol 1^{-1} of sodium hydroxide was prepared in distilled water. 1.0% 1, 3, 5-Trinitrobenzene was prepared in distilled ethanol. Double distilled water was used throughout.

Procedure

Determination of tetracycline hydrochloride in urine 0.50 ml tetracycline hydrochloride from the stock solution was pippitted out made upto the mark with distilled water in 10ml calibrated flask. From this flask, 20-100 µg were taken in a 50ml breakers. To the aliquots, 1 ml of 1.0% 1, 3, 5-trinitrobenzene, 2ml dimethyl sulfoxide and little amount of Dowex 1 x 8 resin beads were added. The solutions are then titrated using a micro-burettle, against 0.01 mol 1^{-1} sodium hydroxide. Transition in colour of the resin beads from light yellow to deep brown signifies the end point. It was prepared with a blank titrated under same set of conditions of observe the sharp distinction in colour change of the resin beads.

Determination of tetracycline hydrochloride in serum

In the same way 0.50 ml tetracycline hydrochloride was pipetted out from the stock solution and diluted up to the volumes with distilled water in a 10ml volumetric flask. From the flask 20-100 µg were taken in a 50ml beakers. To the aliquots, 1 ml of 1.0% 1, 3, 5-trinitrobenzene, 2ml dimethyl sulfoxide and little amount Dowex 1 x 8 resin beads were added. The remaining procedure is the same

as in the case of tetracycline hydrochloride in urine with the same end point.

RESULTS AND DISCUSSION

The calibration curves were constructed by taking solutions of varied amounts of tetracycline hydrochloride in urine and serum. Each solution was titrated with sodium hydroxide, as described inthe experimental part, and the end point recorded. A straight line calibration curves were obtained when the varying amount of tetracycline hydroxide in urine and serum samples were plotted against the volume sodium hydroxide.

Fig. 3.1 shows the calibration curve for tetracycline hydroxhloride in urine. Fig. 3.2 shows the calibration curves for tetracycline hydrochloride in serum. The data used in plotting both the calibration graphs are listed in Table 3.1. The unknown amounts of tetracycline hydrochloride can be completed either from Fig. 3.1 and 3.2 or directly from the equation summarized in Table 3.2. The method was successfully applied to the determination of tetracycline hydrochloride in 10 human urine and 10 serum samples of healthy volunteers.

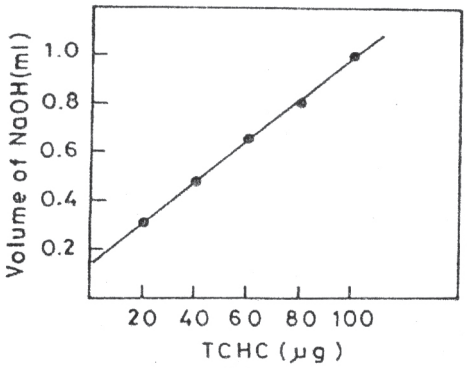

Fig. 3.1: Calibration graph for the determination of tetracycline hydrochloride (TCHC) in urine

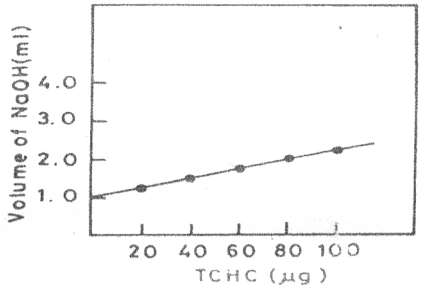

Fig. 3.2: Calibration graph for the determination of tetracycline hydrochloride (TCHC) in serum

After evaluating the developed procedure, the % recovery was found to be 99.90 and 99.47% with a standard deviation of 0.64 and 0.44 µg/ml and % relative standard deviation of 1.06 and 0.74% at 95% confidence level for the determination of tetracycline hydrochloride in urine and serum respectively. The correlation coefficient in both the determination was equal to 1.0000. Therefore the linearity of the calibration graphs and conformity of the systems to Beer's law are proved by the high value of the correlation coefficients of the regression equations.

Spectroscopic observations have shown that tetracycline forms charge-transfer complex with 1, 3, 5-Trinitrobenzene. On addition of sodium hydroxide, the charge-transfer complex between 1, 3, 5-Trinitrobenzene is first formed. When a slight excess of sodium hydroxide added 12, 3, 5-Trinitrobenzene like other polynitro aromatics, is expected to form anionic sigma complexes with bases. This seems all the more likely because dimethyl sulfoxide stabilizes the colour. The deep brown coloured anionic sigma complex is absorbed on the resin beads indicating the end point.

As already mentioned, chiefly due to the importance of tetracycline hydrochloride, it was

Table 3.1: **Data used in plotting the calibration graphs for the determination of tetracycline hydrochloride (TCHC) in urine and serum**

Concentration of	Volume of NaOH (ml)	
TCHC (µg)	Urine	Serum
20	0.30	1.25
40	0.48	1.50
60	0.65	1.75
80	0.80	2.00
100	1.00	2.25

thought in the first instance to develop a titrimetric method for its determination in human urine and blood serum, which can be used in the routine analysis and provides more simple and less time consuming method than the existing ones. Therefore, a complimentary method was devised to fulfill this purpose.

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