# Determination of Sulphadiazine and Tetracycline in Their Mixture by Spectrophotometric Method

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Sulphadiazine and tetracycline have been determined in their mixture by measuring absorbances at 533 nm under acidic conditions (for sulphadiazine) and at 380 nm under basic conditions (for tetracycline).

## INTRODUCTION

A number of methods are available for the determination of sulphadiazine[1-4] and tetracycline[5-8] separately. In this paper we wish to report the determination of sulphadiazine and tetracycline in their mixture.

## EXPERIMENTAL

Sulphadiazine was from sigma chemical company and tetracycline hydrochloride was from Fluka. All the reagents used were of A.R. grade. Doubly distilled water was used for the preparation of solutions. Absorbances were measured on 'Shimadu U.V. Visible Recording Spectrophotometer U.V–2100'.

### Preparation of thiobarbituric acid

2-Thiobarbituric acid (5 g.) was dissolved in 20 ml of N-sodium hydroxide solution and was diluted with 500 ml of water, 250 ml citrate buffer solution was added (prepared by dissolving 35 g. of sodium citrate dihydrate in water, adding 32 ml of conc. hydrochloric acid and·diluting to 250 ml with water) and pH was adjusted to 2.0. The resulting solution was stored in a refrigerator.

### Calibration curve for sulphadiazine

Sulphadiazine (5 mg) was dissolved in 10 ml N-hydrochloric acid and 90 ml water to give a solution of sulphadiazine (conc. 0.05 mg/ml).

A 5 ml of above solution was diluted to 10 ml with 0.1 N hydrochloric acid so that the concentration of sulphadiazine is 25 $\mu$g/ml. One ml solution of sulphadiazine (conc. 25 $\mu$g/ml) was taken in a 10 ml flask and diluted with thiobarbituric acid solution. In another flask 1 ml of 0.1 N HCl was taken and diluted with thiobarbituric acid solution. Both the flasks were placed on water bath and when the solutions had attained temp. of the bath (60°C), flasks were stoppered and allowed to stand for

'1 hr. Thereafter stoppers were removed, allowed to cool and water was added to compensate for evaporation during heating. Absorbances of standard solutions were measured at 533 nm using 1 cm cells with blank solution in the comparison cell. Absorbances at 533 nm were measured for different concentration of sulphadiazine solution (Table 1) and a calibration curve was drawn (Fig. 1).

TABLE 1

MEASUREMENT OF ABSORBANCES FOR SULPHADIAZINE SOLUTIONS

| Sl. No. | Preparation of solutions | | Final con-centration of sulpha-diazine ($\mu$g/ml) | Absorbances at 533 nm |
| | Volume of sulpha-diazine (0.05 mg/ml) | Volume of HCl (0.1N HCl) | | |
|---|---|---|---|---|
| 1. | 1 ml | 9 ml | 0.5 | 0.172 |
| 2. | 2 ml | 8 ml | 1.0 | 0.361 |
| 3. | 3 ml | 7 ml | 1.5 | 0.531 |
| 4. | 4 ml | 6 ml | 2.0 | 0.690 |
| 5. | 5 ml | 5 ml | 2.5 | 0.850 |
| 6. | 6 ml | 4 ml | 3.0 | 0.985 |
| 7. | 7 ml | 3 ml | 3.5 | 1.241 |

**Calibration curve for tetracycline**

Tetracycline hydrochloride (25 mg) was dissolved in 250 ml water. A 15 ml aliquot was transferred to a 100 ml graduated flask, 70 ml water was added, then 5.0 ml of 5 N sodium hydroxide was added and was finally diluted to 100 ml. with water to give tetracycline solution (conc. 15 $\mu$g/ml). Six minutes after the addition of alkali, absorbance was measured at 380 nm with water as blank. Absorbances at 380 nm were measured for various concentrations of tetracycline solution (Table 2) and a calibra-tion curve was drawn (Fig. 2).

**Determination of sulphadiazine and tetracycline in their mixture**

(i) A mixture of 5 mg of sulphadiazine and 5 mg of tetracycline hydro-chloride was dissolved in 10 ml N hydrochloric acid and 90 ml water to give a solution of the mixture (conc. of sulphadiazine 0.05 mg/ml).

The above solution was further diluted with. 1 N Hydrochloric acid and then with thiobarbituric acid as mentioned in the procedure for the calibration curve of sulphadiazine. The absorbance measured for this
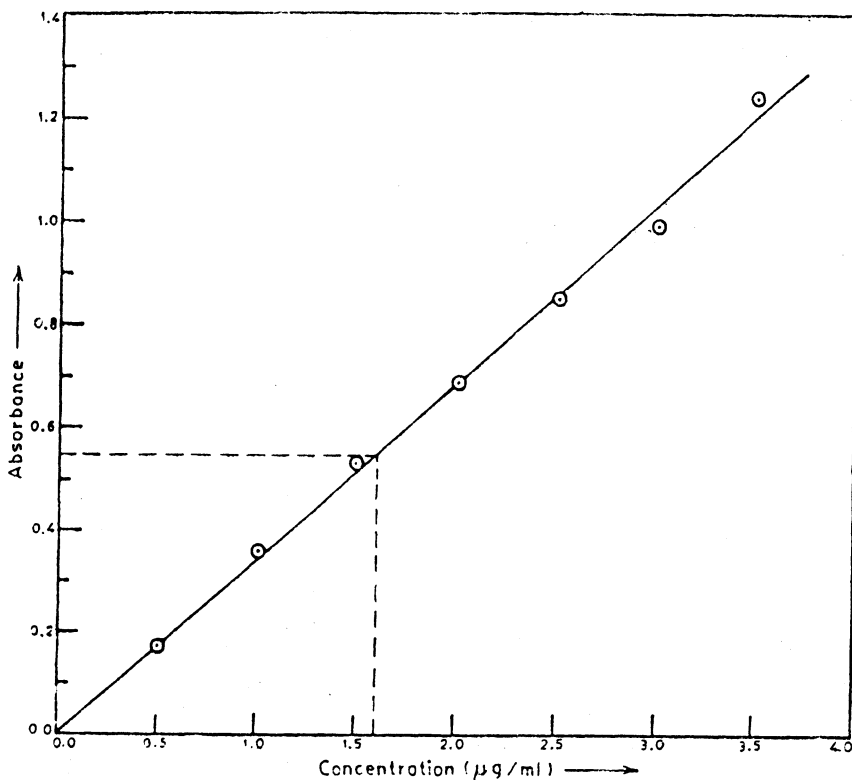
Fig. 1.   Calibration curve for the determination of sulphadiazine.

TABLE 2

MEASUREMENT OF ABSORBANCES FOR  TETRACYCLINE SOLUTIONS

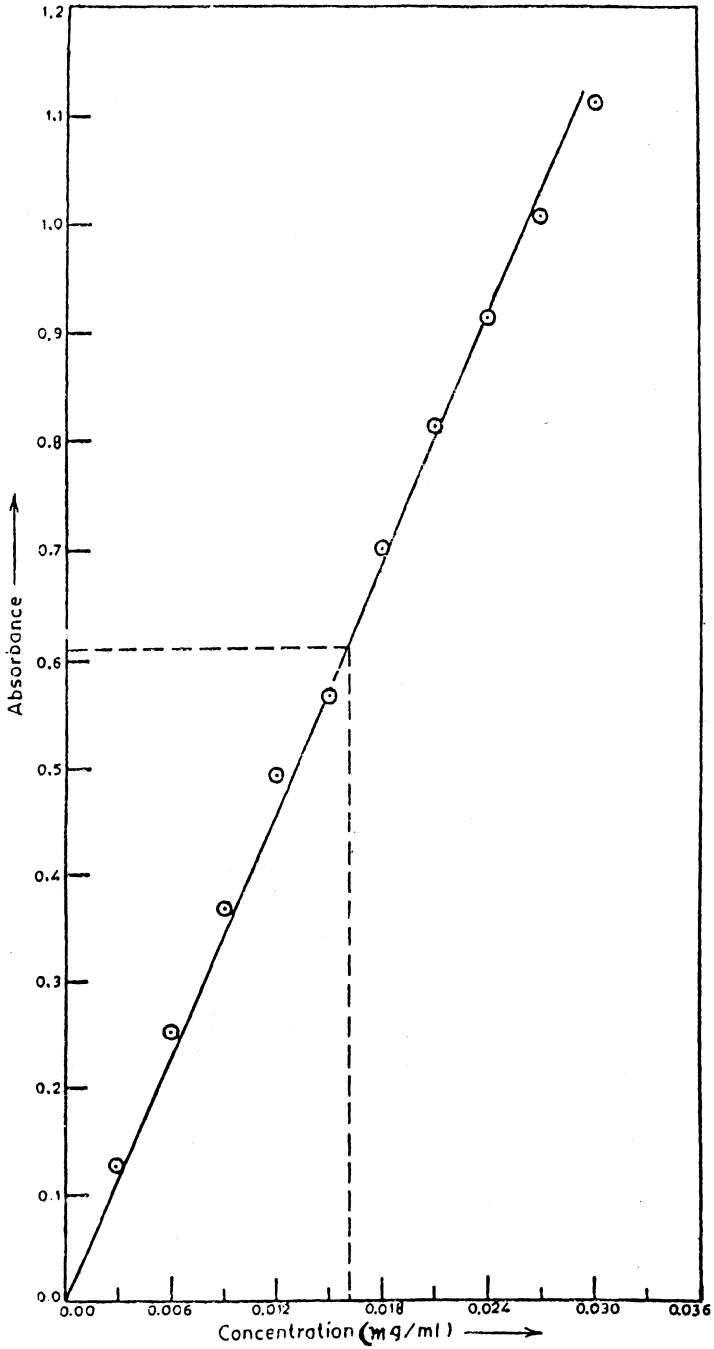| Sl. No. | Preparation of solutions | Final concen-tration of tetracycline (mg/ml) | Absorbance at 380 cm |
|---|---|---|---|
| | 15 ml of solution having  following con-centrations mg/ml were diluted to 100 ml | | |
| 1. | 0.02 | 0.003 | 0.125 |
| 2. | 0.04 | 0.006 | 0.252 |
| 3. | 0.06 | 0.009 | 0.368 |
| 4. | 0.08 | 0.012 | 0.493 |
| 5. | 0.10 | 0.015 | 0.567 |
| 6. | 0.12 | 0.018 | 0.699 |
| 7. | 0.14 | 0.021 | 0.812 |
| 8. | 0.16 | 0.024 | 0.914 |
| 9. | 0.18 | 0.027 | 1.039 |
| 10. | 0.20 | 0.03 | 1.120 |

Fig. 2.   Calibration curve for the determination of tetracycline.

solution at 533 nm comes out to be 0.900. For this absorbance the corresponding concentration of sulphadiazine from the calibration curve (Fig. 1) comes out to be 2.6 $\mu$g/ml. whereas the actual conc. was 2.5 $\mu$/ml.

(ii) Tetracycline hydrochloride (10 mg) and sulphadiazine (10 mg) was dissolved in 100 ml water. A 15 ml aliquot was transferred to a 100 ml graduated flask, 70 ml water, 5 ml 5 N sodium hydroxide was added and was finally diluted to 100 ml. (conc. of tetracycline 15 $\mu$g/ml). Absorbance at 380 nm was measured by following the procedure mentioned for the calibration curve of tetracycline. The absorbance at 380 nm comes out to be 0.610. For this absorbance the corresponding concentration from the calibration curve (Fig. 2) comes out to be 16 $\mu$g/ml. whereas the actual conc. was 15 $\mu$g/ml.

## RESULTS AND DISCUSSION

This method is based on the observation that sulphadiazine in the presence of thiobarbituric acid shows absorbance at 533 nm and tetracycline hydrochloride in the presence of sodium hydroxide shows absorbance at 380 nm. In a mixture of tetracycline hydrochloride and sulphadiazine, sulphadiazine can be determined under acidic conditions (by measuring absorbance at 533 nm) and tetracycline does not interfere whereas tetracycline can be determined under basic conditions (by measuring absorbance at 380 nm.) and sulphadiazine does not interfere.

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