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## Colorimetric determination of *Thiamine Hydrochloride* using *Alizarin Brilliant Violet R*.

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**A new colorimetric method for the determination of thiamine hydrochloride is developed. It is based on the formation of coloured ion-pair complex between thiamine hydrochloride and alizarin brilliant violet R in the aqueous phase at pH 4.5 extractable into chloroform which obeys Beer's law in the range of 2-16 mcg/ml.**

SEVERAL colorimetric procedures are reported in literature<sup>1-10</sup> for the determination of thiamine hydrochloride. The present method is based on the formation of coloured ion pair complex between thiamine hydrochloride and alizarin brilliant violet R in the aqueous phase at pH 4.5 extractable into chloroform in which it is stable for 4 hours and is measured at 575 nm.

Chemito 2500 U.V-Visible scanning spectrophotometer, aqueous solution of alizarin brilliant violet R (0.2% w/v) and potassium hydrogen phthalate-sodium hydroxide solution (pH 4.5) were used.

About 10 mg. of thiamine hydrochloride (pure or formulations) was accurately weighed and dissolved in distilled water to make 100 ml. which gave

a concentration of 100 mcg/ml of thiamine hydrochloride.

In a 100 ml separating funnel containing 25 ml of chloroform, aqueous solutions of 0.5 ml - 4 ml of thiamine hydrochloride (50 mcg - 400 mcg), 2 ml of alizarin brilliant violet R (0.2%), 6 ml of potassium hydrogen phthalate-sodium hydroxide buffer (pH 4.5) and appropriate volume of water to bring the total volume to 10 ml were added. The separator was shaken for 2 min and set aside for separation. The chloroform layer was separated and the absorbance of the blue coloured chloroform extract was measured at 575 nm against a reagent blank. The standard graph was plotted using absorbance versus concentration.

The sample solutions were also treated in a similar manner as in the standard curve. The amount

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**Table 1**  
**Result of analysis of Thiamine Hydrochloride in Pharmaceutical formulations**

Sl. dosage form No.	Labelled amount mg.	Contents of thiamine		Percent recovery (%)
		Reported method mg.	Proposed method mg.	
<b>Injection (1ml)</b>				
1.	10	11.6	11.7	99.60
2.	2	2.41	2.39	99.80
<b>Tablets</b>				
3.	2	1.86	1.88	99.90
4.	10	11.25	11.20	99.86
<b>Capsules</b>				
5.	10	9.86	9.91	99.75
6.	5	5.15	5.21	99.80
<b>Syrups</b>				
7.	1	1.05	1.03	99.80
8.	2.5	2.85	2.83	99.86

Excess values obtained reflect the averages of Vitamins added.

of samples were computed from the standard calibration graph of thiamine hydrochloride.

The stoichiometric relationship of drug: dye has been found to be 1:1 through the slope ratio method. Beer's law limits (mcg/ml), molar extinction coefficient ( $1 \text{ mole}^{-1}, \text{ cm}^{-1}$ ) and Sandell's sensitivity ( $\text{mcg}/\text{cm}^2/0.001$  absorbance Unit) of thiamine hydrochloride were found to be 2-16 mcg,  $1.18 \times 10^4$  and 0.029 mcg/ml, respectively. The precision of the method was checked by measuring the absorbance of five samples, each containing fixed amount of thiamine hydrochloride. The relative standard deviation is 1.21%. To evaluate the validity and reproducibility of the method, known amounts of pure drug were added to the previously analysed samples and the mixtures were analysed by the proposed method. The recovery ranged between 99.4% to 99.9%. The proposed method has been applied to commercial formulations. The results obtained by

the proposed and reported methods are presented in Table 1. These results indicate that the method is accurate, precise and reproducible.

The method is simple, rapid and applicable to various formulations. There is no interference of excipients and other vitamins. The coloured complex is stable for 4 h. and can be used in routine determination of thiamine in Pharmaceutical formulations.

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## Correction of raw dissolution data for loss of drug and volume during sampling

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**The communication reports the derivation of relationships that can be directly applied to correct raw dissolution data for loss of drug and/or volume reduction during manual sampling in with replacement and without replacement studies.**

**D**URING multi-point dissolution studies, a small aliquot is withdrawn at predetermined time intervals from the dissolution medium. The withdrawal of the sample not only causes a progressive decrease in the total dissolution medium, it also results in a cumulative loss of drug with each sample. Both these effects cause an error in the data obtained during without-replacement (WOR) studies, where the sample drawn is not replaced by an equal quantity of fresh medium. In the with-replacement (WR) studies, the volume is adjusted back to the original after each sampling and the error accrues in this case from the loss of drug only.

The dissolution data, in case of both WOR and WR studies, is hence required to be corrected for the error due to the loss of drug and/or change in the total medium volume. The correction can be

applied mathematically to either raw absorbance (or concentration) data that are obtained directly on analysis of dissolution samples, or the derived amount (or percent) released values. The application of correction to the former is, however, advantageous as it provides freedom to calculate the derived amount, fraction or percent drug released values to the requirements of the user.

The factors for correction of derived values of amount (or percent) drug dissolved in WOR studies have been reported in literature<sup>1</sup>. In this communication we extend the reported relationships to calculate factors for correction of raw absorbance (or concentration) data obtained using either WOR or WR studies.

The formula described for calculation of corrected amount of drug dissolved, after the nth sample (1) is as follows:

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